ROLE OF EXPRESSION OF P63 AND CALPONIN IN GASTRO-INTESTINAL TRACT CARCINOMAS

Dissertation submitted in

Partial fulfillment of the requirements

for the award of

M.D. DEGREE

in

PATHOLOGY – BRANCH III

THE TAMILNADU

DR. M.G.R. MEDICAL UNIVERSITY

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DECLARATION

I hereby declare that the dissertation entitled **"ROLE OF EXPRESSION OF p63 AND CALPONIN IN GASTRO-INTESTINAL TRACT CARCINOMAS"** was done by me in the Department of Pathology, Chengalpattu Medical College from June 2014 to June 2017 under the guidance and supervision of **Dr. K.R. MOHAN, M.D**., Associate Professor, Department of Pathology, Chengalpattu Medical College.

This dissertation is submitted to the Tamil Nadu Dr.MGR Medical University, Chennai towards the partial fulfillment of the requirements for the award of M.D. Degree in Pathology.

I have not submitted this dissertation on any previous occasion to any University for the award of any degree.

Dr. R. KAMARAJ

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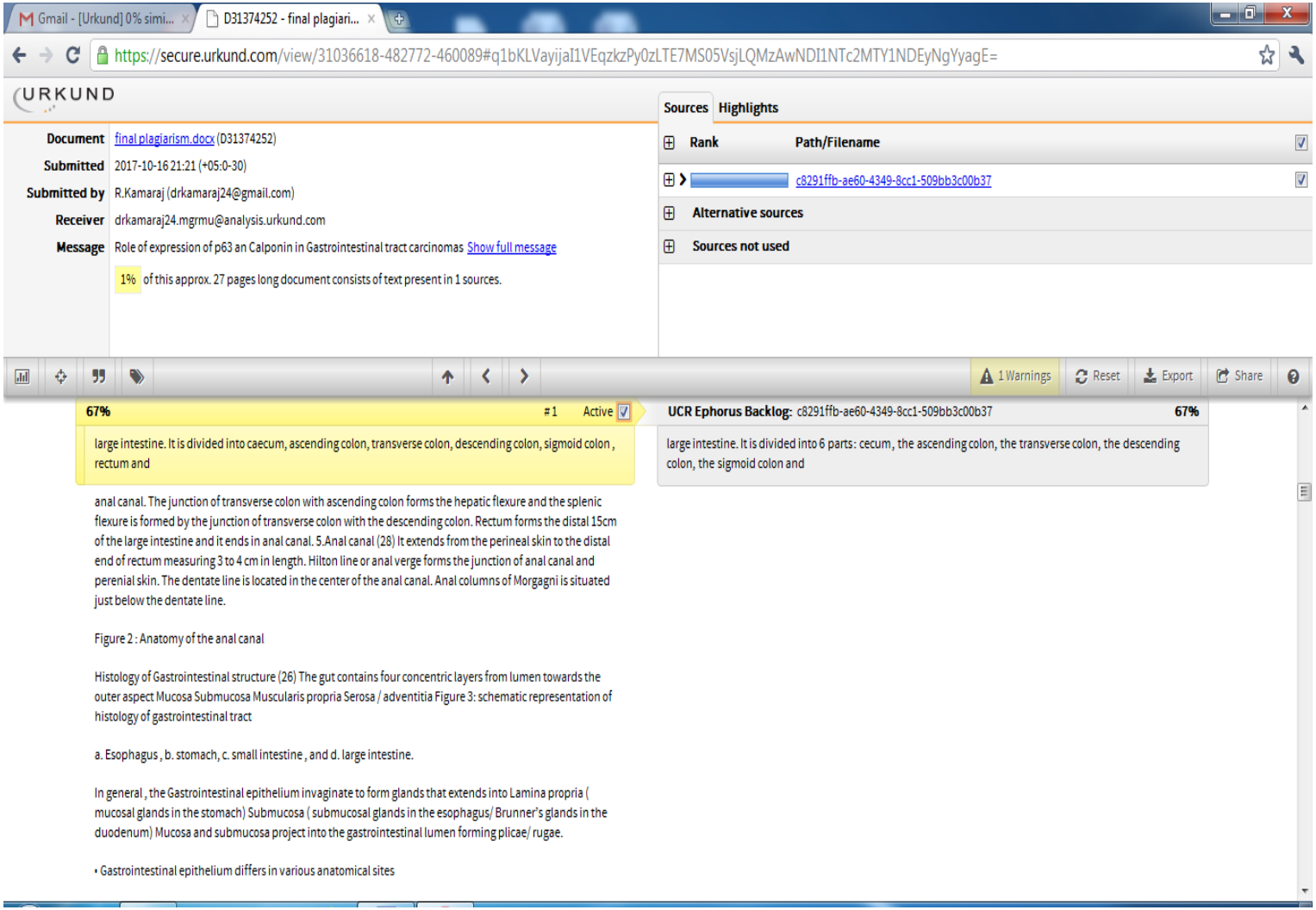
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large intestine. It is divided into 6 parts: cecum, the ascending colon, the transverse colon, the descending colon, the sigmoid colon and
anal canal. The junction of transverse colon with ascending colon forms the hepatic flexure and the splenic flexure is formed by the junction of transverse colon with the descending colon. Rectum forms the distal 15cm of the large intestine and it ends in anal canal. 5.Anal canal (28) It extends from the perineal skin to the distal end of rectum measuring 3 to 4 cm in length. Hilton line or anal verge forms the junction of anal canal and perenial skin. The dentate line is located in the center of the anal canal. Anal columns of Morgagni is situated just below the dentate line.
Figure 2 : Anatomy of the anal canal
Histology of Gastrointestinal structure (26) The gut contains four concentric layers from lumen towards the outer aspect Mucosa Submucosa Muscularis propria Serosa / adventitia Figure 3: schematic representation of histology of gastrointestinal tract
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INTRODUCTION

Gastrointestinal tract malignancies constitutes about 27% of total cancers and 20% of all cancer deaths worldwide with highest burden of 62% in Asia (1)(7).As GIT malignancies are on the rise in developing countries like india, it is essential to classify the type with histopathological, immunohistochemical molecular genetic features for prognostic significance and for giving target based treatment. Our aim of this study is to evaluate the expression of immunohistochemical markers P63 and Calponin in the gastrointestinal tract malignancies and to correlate it with the histopathological features. Immunohistochemistry plays an important role in grading of gastrointestinal carcinomas. P63 and calponin are the new markers tried in gastrointestinal tract malignancies and the results were promising.

P63 is nuclear marker(2), useful in the prognostic evaluation of esophageal carcinomas,poorly differentiated gastric carcinomas(90), poorly differentiated colorectal carcinomas and anal squamous cell carcinomas. Intense positivity (>50% of tumor cells) leads to poor prognosis.

Calponin is cytoplasmic staining marker (3)and in some studies it was found that its high expression in the colonic tumor blood vessels inhibits metastases by contraction of vascular smooth muscle cells.

Both these immunohistochemical markers will be helpful as a tool in predicting the prognosis of patients with gastrointestinal tract malignancies and play a role in targeted therapy in near future.

AIMS AND OBJECTIVES

1. To correlate the age, sex and anatomical site of distribution of gastrointestinal tract malignancies

2. To study the histopathological features of gastrointestinal tract malignancies

3. To study the role of expression of immunohistochemaical markers P63 and calponin in gastrointestinal tract malignancies

4. To correlate the histopathological features with expression of immunohistochemical markers P63 and calponin.

REVIEW OF LITERATURE

Cancer is one of the leading causes of deaths worldwide. The mortality due to cancer in India is projected to increase because of population growth, lifestyle changes, urbanization, increasing life expectancy and also due to industrialization,. The digestive tract is a major site of cancer in humans. There are great differences in incidence among the anatomical sites of the gastrointestinal tract from the esophagus to the anus. The most prevalent of these is gastric cancer in the Eastern world and colorectal cancer in the Western world. Approximately 630,000 deaths occur due to colorectal cancer worldwide, accounting for 7 – 8% of all cancer deaths. Gastric cancer accounts for approximately 800,000 deaths each year worldwide. In India, esophageal cancer is highest in northeastern region, for both males and females. Gastric cancer is also prominent problem in northeastern and southern states of the Indian subcontinent. In India,(4) the Squamous cell carcinoma of the anal canal is an under recognized malignancy.

EPIDEMIOLOGY OF GASTROINTESTINAL TRACT MALIGNANCIES

- **Esophageal cancer in India(5)**

Fourth common cause of cancer related deaths in India. Eighth common cause worldwide. Squamous cell carcinoma accounts for 80% but now adenocarcinoma on the rise due to changes in the life style. Predisposing

factors are tobacco, alcohol & poor nutrition. countries with high HDI (Human Developmental Index) are having increased adenocarcinoma incidence(6). In U.S adenocarcinoma risk increased to 400% in the past 25 years. In India with low HDI, squamous cell carcinoma incidence is more, accounting for approximately 47,000 new cases detected per year with mortality of 42,000 per year in India(7).

- **Gastric cancer(8)**

Fifth commonest cancer worldwide with male to female incidence of 2:1.Gastric cancers are mainly divided into cardia & non- cardia cancers. Helicobacter pylori plays an important role in non – cardiac cancers, two to three fold increased risk compared to non H. pylori. H.pylori plays a role mainly in gastric adenocarcinomas due to Cag A, virulence factor.EBV association is also found in some of the cases. Predisposing factors are tobacco, alcoholic drinks, salted foods, obesity & processed meat. Diet & lifestyle plays an important role in gastric cancers. Increased salt intake damages gastric mucosa & leads to cell proliferation. Pickled foods, spicy foods, chilly foods, increased temperature foods, smoked dried salted foods are some of the predisposing factors. Increased incidence in Kashmir due to consumption of salted tea was found in one study. Low starch diet, garlic, onion & selenium are protective factors. Reheated foods, oils & refrigeration are not risk facors for gastric cancer.

- **Small intestinal cancers(9)**

The incidence of small intestinal cancers is less compared to colorectal malignancies. several mechanisms play a role for this, 1.much quicker transit time of food in small intestine compare to large intestine. (increased peristaltic ring contractions in small intestine compared to latter) leads to shorter time of exposure of mucosa to carcinogens). 2.lower bacterial load in small intestine & therefore decreased concentration of potential carcinogens from bile acid breakdown(10). 3. High IgA expression in small intestine confers protection against lymphoma. 4. Generation of less ROS (reactive oxygen species) in small intestine compared to colon(11). Predisposing conditions for small intestinal cancers are inflammatory bowel disease (Crohn's disease and ulcerative colitis), celiac disease, familial adenomatous polyposis, Peutz – Jeghers syndrome. Associated other malignancies are Hereditary non polyposis colorectal cancer(HNPCC)(12).

- **Colorectal cancer(13)**

It is the third most common cancer in men and second most common cancer in women. Colorectal cancer related deaths are estimated to be approximately 60800 worldwide accounting for 8 % of all cancer deaths. In India, the annual incidence rates for colon cancer and rectal cancer are 4.4 and 4.1 per 100,000 respectively. In a recent study, they found out that pattern of genomic alterations in colon and rectal tissues were similar regardless of origin and anatomical location and therefore tumors of colon and rectum can be

grouped together. 24 genes were found mutated in addition to genes identified priorly (APC, TP53, KRAS, and PIK3CA). The new genes were SOX9, FAM123B/WTX, ERBB2 and IGF2(14).

Risk factors for colorectal cancers

Divided into genetic and environmental or life style related factors

Genetic factors

Familial adenomatous polyposis (FAP) and its variants (Turcot, Gardner and attenuated FAP) and MYH associated polyposis are most common. HNPCC/ Lynch syndrome constitutes the non polyposis colonic category.

FAP presents as multiple colonic adenomatous polyps in childhood transforming into malignancy at an average age of 45 years due to germline mutation of of APC gene on chromosome 5. Attenuated FAP has same genetic mutation as FAP but characterized by later average age presentation of colorectal malignancies and fewer adenomas compared to former(15).

Lynch syndrome (HNPCC), an autosomal dominant condition is due to defect in one of the MMR(mismatch repair) genes, namely MLH 1, MSH 2, HMSH6,or PMS2. Characteristic feature of this is early average age of onset of colorectal malignancy and right sided predominant colonic lesions and its association with thyroid, breast and gynaecological malignancies(16).

Environmental risk factors(17), (18),(19)

Older age group and males are having increased risk of colorectal cancer. Increased risk was noted among African American population. Long term immunosupression, especially after renal transplantation posseses same relative risk, but aged 20-30 years older. Some other important risk factors are insulin like growth factors (diabetes with insulin resistance), alcohol consumption(especially with positive family history),ulcerative colitis, processed meat and fresh red meat consumption, Obesity, cigarette smoking,Androgen deprivation therapy (orchidectomy),Acromegaly,History of cholecystectomy and Ureterocolic anastomosis.

Other major associations with conflicting evidences

Presence of coronary heart disease,decreased fibre and fruit intake, history of prostrate cancer radiotherapy, history of treatment for Hodgkin's lymphoma and decreased physical activity.

- **Anal cancer**

Comprises 2.5% of all gastrointestinal malignancies but incidence is on the rise for past 30 years(20). Increased incidence is seen in the following - females, infection with Human papilloma virus (HPV)(20), infection with human immunodeficiency virus, lifetime number of sexual partners, genital warts, cigarette smoking, receptive anal intercourse. In these perspective anal

canal malignancies were considered similar to genital malignancies rather than gastrointestinal tract malignancies.

In 1960's, anal malignancies were thought to be due to chronic perianal inflammation and treated routinely by abdominoperineal resection warranting a permanent colostomy.But now substantial progress has been made in the treatment of anal malignancies. Now it has been found out that HPV infection is associated in most of the cases and cure of anal malignancy is possible with sparing of anal sphincter(21).

Embryology of the gastrointestinal tract

Gut system extends from the buccopharyngeal membrane to the cloacalmembrane and is divided into pharyngeal gut, foregut, midgut and hindgut. Epithelium of the digestive system originate in the endoderm, while the connective tissue, muscular component and peritoneal components originate in the mesoderm.(22)

Endoderm and ectoderm contact one another in the 2- to 4-week embryo. The endoderm, which forms the roof of the yolk sac, gives rise to the future gut, creating the majority of the epithelial lining of the GI tract, biliary passages, liver, and pancreas.

1. Esophageal Development(23)

The cranial part of the primitive foregut gives rise to esophagus, recognizable at the third week of gestation as an annular constriction located between the stomach and pharynx, elongates and grows in a cephalad direction becoming tubular. Cephalad parts of both the esophagus and trachea lie within a single common tube. Primitive mesenchyme grows into forming septum, eventually separating the esophagus and trachea.

2. Stomach development(24)

The stomach develops from a fusiform foregut swelling at approximately 4 weeks of gestation. It originates in the neck and descends into the abdomen during the next 8 weeks. The enlarging thoracic contents push the stomach caudally. The gastric curvature develops during the 6th to 7th fetal week. The first differentiated cell type to appear is the mucous neck cell, which acts as a progenitor for the other cell types. Gastric glands begin to develop at 11 to 14 weeks. Parietal cells appear by 9 to 11 weeks. Endocrine cells begin to appear at the second week of fetal life; a full spectrum of endocrine cells is present by week 11. Mesoderm surrounding the stomach differentiates into the gastric connective tissue and the muscularis propria by the end of the second fetal month. The muscularis mucosae forms by the 20th week.

3. Small intestine development(25)

The duodenum distal to the bile duct, and the jejunum, ileum, cecum, ascending colon, and proximal one half to two thirds of the transverse colon derive from the midgut and are supplied by the superior mesenteric artery. Villous formation with mesenchymal infiltration into the villous core begins at the ninth gestational week. Between 9 and 10 weeks, the stratified epithelium converts to a simple columnar epithelium (13). Villi are long and tapering by 20 weeks and the muscle coats are obvious at this time. The epithelium finishes its morphologic differentiation into enterocytes, goblet cells, endocrine cells, and Paneth cells in the 4 to 5 days prior to birth (16).

4. Colon, rectum and anal canal development(26)

Embryologic midgut gives rise to the proximal colon, including the cecum, the ascending colon, and the first two thirds of the transverse colon. The rest of the colon and rectum derive from the embryologic hindgut.. The midgut returns to the abdominal cavity during the third fetal month. The small intestine enters the abdomen first, followed by the cecum. During re-entry, the gut rotates an additional 180 degrees, so that the cecum comes to lie in the right upper quadrant, hindgut develops into the left transverse colon, descending colon, sigmoid colon, rectum, and upper anal canal. The distal hindgut enters the cloaca, the structure that ultimately forms the anal canal and the urogenital structures.

Structure of gastrointestinal tract

Gastrointestinal tract comprises the following structures:

Esophagus

Stomach

Small intestine (duodenum, jejunum and ileum)

Large intestine (ascending colon, transverse colon, descending colon and sigmoid colon)

Appendix

Rectum and anal canal.

Accessory organs of gastrointestinal system are

Liver and gall bladder

Pancreas

Figure 1. Anatomy of human Gastrointestinal tract

Anatomy of gastrointestinal tract

1. Esophagus (27)

Esophagus extends from cricopharyngeus muscle to gastroesophageal junction which forms the upper and lower esophageal sphincters respectively. It is a round muscular tube measuring 25cm in length. The lower esophageal sphincter is about 40 cm from the inscisor teeth. Lymphatics from the upper third of esophagus drains into cervical nodes, middle third drains into paraesophageal and paratracheal mediastinal nodes, lower third into aorta and celiac nodes.

2. Stomach(28)

Grossly, the stomach is divided into five regions : cardia, fundus,corpus or body, antrum and pylorus. Lesser curvature forms superomedial margin, greater curvature forms the inferolateral margin. Incisura is the notch in lesser curvature formed between the junction of corpus and the antrum. Mucosal folds present internally forms the rugae

3. Small intestine(28)

Divided into three parts: duodenum, jejunum and ileum.Duodenum is mostly retroperitoneal and fixed part of the small intestine. Duodenojejunal junction is marked anatomically by the ligament of treitz (the site at which the bowel becomes unfixed). Kerckring folds are transverse mucosal folds present internally. Peyers patches are lymphoid follicles present in the terminal ileum on its antimesenteric side with its long axes parallel to the bowel.

4. Large intestine(28)

The terminal 1 to 1.5m of the gastrointestinal system is formed by large intestine. It is divided into caecum, ascending colon, transverse colon, descending colon, sigmoid colon, rectum and anal canal. The junction of transverse colon with ascending colon forms the hepatic flexure and the splenic flexure is formed by the junction of transverse colon with the descending colon. Rectum forms the distal 15cm of the large intestine and it ends in anal canal.

5. Anal canal(28)

It extends from the perineal skin to the distal end of rectum measuring 3 to 4 cm in length. Hilton line or anal verge forms the junction of anal canal and perenial skin. The dentate line is located in the center of the anal canal. Anal columns of Morgagni is situated just below the dentate line.

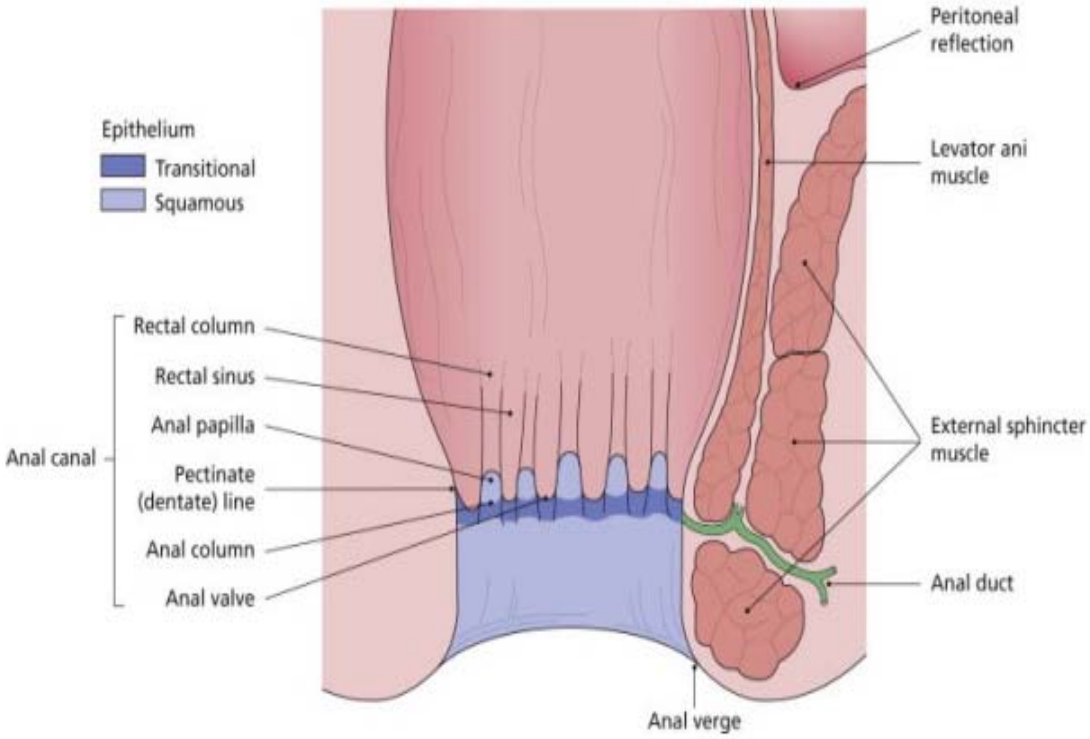


Figure 2 : Anatomy of the anal canal

Histology of Gastrointestinal structure (26)

The gut contains four concentric layers from lumen towards the outer aspect

Mucosa

Submucosa

Muscularis propria

Serosa / adventitia

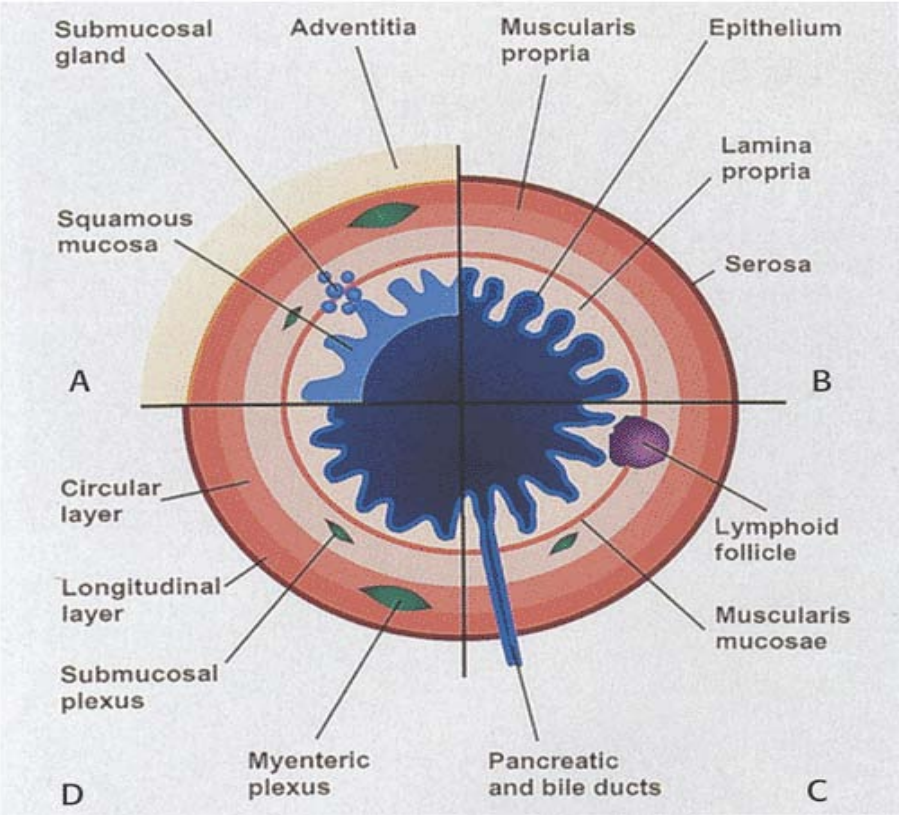


Figure 3: schematic representation of histology of gastrointestinal tract

a. Esophagus, b. stomach, c. small intestine, and d. large intestine.

In general, the Gastrointestinal epithelium invaginate to form glands that extends into

Lamina propria (mucosal glands in the stomach)

Submucosa (submucosal glands in the esophagus/ Brunner's glands in the duodenum)

Mucosa and submucosa project into the gastrointestinal lumen forming plicae/ rugae.

- Gastrointestinal epithelium differs in various anatomical sites

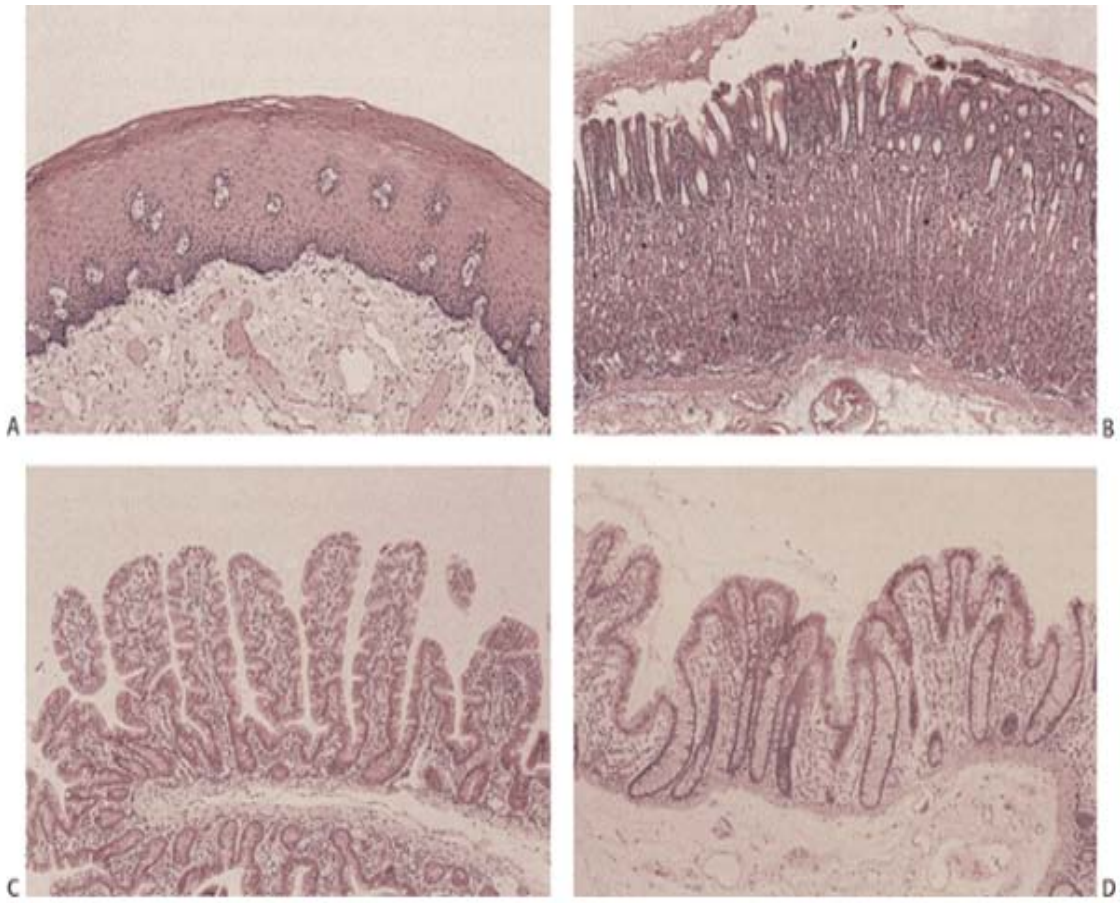


Figure : 4 Histology of Gastrointestinal tract epithelium

A. Squamous epithelium lines the esophagus and anus.

B. In stomach, the lining columnar epithelium comprises surface epithelium, pits and glands.

C. In small intestine, the mucosa comprises crypts and villi and intestinal epithelial cells.

D. Colon contains similar type of cells but it lacks villi.

Histology of the gastrointestinal tract

- Esophagus

Is lined by squamous epithelium, except at its distal end. The squamous lining of esophagus protects it from damaging effects of undigested food particles over its surface. The squamous mucosa contains three components: Squamous epithelium, lamina propria, and a thick muscularis mucosae. The squamous epithelium consists of nonkeratinizing stratified squamous epithelium. In esophagus, epithelial cell renewal takes an average of 7 days (26).

- Squamo columnar junction (29)

The normal squamocolumnar junction (SCJ) lies at the level of the diaphragm. Current criteria for definition of GEJ is of mainly with gross or endoscopic junction, the point of flaring of the esophagus, the proximal limit of gastric rugal folds and presence of lower esophageal sphincter. So as per above criteria the GEJ is proximally displaced above the Z line or squamocolumnar junction. Variability in the extent of the cardiac mucosa likely reflects the presence of underlying disorders such as GERD or Helicobacter pylori gastritis (25) and suggests that the area of the GEJ is a dynamic structure that may change over time.

- Stomach(26)

The epithelial compartments of stomach is divided into three components namely gastric pits and surface lining epithelium, mucous neck region and glands. The Stomach is divided into cardiac, oxyntic and pyloric areas based on glandular components. The 80% of proximal stomach is constituted by oxyntic mucosa and it secretes acid and pepsinogen and the distal 20% of stomach is constituted by antropyloric mucosa and has mucous secreting glands and endocrine cells. The lamina propria contains loose connective tissue rich in immunocompetent cells and muscularis mucosae Muscularis mucosae contains smooth muscle cells arranged predominantly in circular fashion. The Muscularis Propria(26) contains two layers of muscle fibres, the inner muscular layer (the circular layer) and the outer muscular (longitudinal layer) named due to the arrangement of muscle fibres.. The outermost layer is called adventitia or serosa of loose connective tissue containing fat, collagen, and elastic tissues.

- Small intestine(26)

In small intestine, the epithelial lining is uniquely suited to the further digestion and absorption of nutrients along a gradient from duodenum to the ileum. Contains mainly two components, crypts of Lieberkuhn and villi. Villi height : crypt is 3:1 in adults and 2:1 in children. The lining epithelium contains heteologous population of cells, enterocytes (absorptive cells), goblet cells, endocrine cells, tuft cells, cup cells and M cells. The lamina propria

forms the mucosal interglandular tissues. It appears as a delicate, loose, connective tissue containing lymphocytes, plasma cells, eosinophils, rarely neutrophils, and mast cells. Majority of plasma cells secrete IgA. Lamina propria also contains large numbers of macrophages, which play important roles in mucosal immunity, antigen presentation, elimination of exogenous pathologic antigens or organisms, immunoglobulin production, and immunoregulation (30). The gut-associated lymphoid tissue (GALT) primarily lies within the lamina propria. It is distributed diffusely or appears as solitary or aggregated nodules, which in the ileum are called Peyer patches. Larger aggregates contain germinal centers.

- Large intestine(31)

Mucosa is smooth and lacks villi. It contains goblet cells, absorptive cells, tuft cells, endocrine cells and paneth cells (especiaaly in the caecum), the ratio of absorptive cells to goblet cell is 3:1. Goblet cells secretes mucin into the lumen. In the cecum and in parts of the colon, the longitudinal muscle is extremely attenuated except in the regions where it forms thick cords (i.e., the taeniae coli).The musculature also contains the interstitial cells of Cajal (ICCs). ICCs act as pacemakers for the GI muscle (13), facilitate active propagation of electrical events, and also mediate neurotransmission. Anus(26)

- Anus

The mucosa is being protected by squamous epithelium from the damaging effects of the passage of solid waste. The anal mucosa is divided into four zones 1.coloectal zone 2.transitional zone 3.smooth zone and 4. Distal zone.

GASTROINTESTINAL TUMORS

ESOPHAGEAL TUMORS

WHO Histologic Classification of Esophageal Tumors(32)

I. Epithelial tumors

Benign

-Squamous cell papilloma

Malignant

-Intraepithelial neoplasia

Squamous

Glandular (adenoma)

Carcinoma

1. Squamous cell carcinoma
    a. Verrucous (squamous) carcinoma
    b. Basaloid squamous cell carcinoma
    c. Spindle cell (squamous) carcinoma

2. Adenocarcinoma

3. Adenosquamous carcinoma

4. Mucoepidermoid carcinoma

5. Adenoid cystic carcinoma

6. Small cell carcinoma

7. Carcinoid tumor

II. Nonepithelial tumors

Benign	**Malignant**
Leiomyoma	Leiomyosarcoma
Lipoma	Rhabdomyosarcoma
Granular cell tumor	Kaposi's sarcoma
	Malignant melanoma

-Gastrointestinal stromal tumor

Benign

Uncertain malignant potential

Malignant

-Others

-Secondary tumors/ metastatic tumors

Esophageal malignancies

Squamous cell carcinoma accounts for majority of esophageal malignancies and incidence differs worldwide. The risk factors are smoking, alcohol abuse, diet, genetic factors and HPV (33). Achalasia cardia (34), tylosis Palmaris et plantaris (35), a genetic disorder characterized by hyperkeratosis of palms and soles. Peak incidence – 55 to 65 years with M: F ratio of 3-7:1. Most common site – middle third of esophagus (60%), compared to lower third (30%). Usually presents at advanced stage with poor prognosis.

- Pathological features

1. Gross features

These tumors can present as irregular deep ulcers with nodularity in the margins or exophytic fungating mass or as mural thickenings.submucosal infiltration can undermine adjacent normal mucosa leading to luminal narrowing similating a benign stricture and this submucosal spread is not seen on gross inspection.

2. Microscopic features

Any degree of differentiation can occur. Well differentiated lesions exhibits mild nuclear atypia, pleomophism and adundant keratinisation. In poorly differentiated tumors keratinisation is sparse with significant cytological abnormalities.

- Superficial squamous cell carcinoma

Confined to mucosa and submucosa irrespective of nodal status(36) these cases were first detected in patients who underwent screening for esophageal malignancies in Japan and China.In United states and Western Europe, it accounts for upto 22% of resected esophageal squamous arcinomas(37). Grossly presents as eroded mucosal depressions, thin plaques or even sometimes inapparent on gross inspection. Microscopically it consists of invasive nests with irregular borders within the lamina propria or submucosa with variable tumor differentiation. These are potentially curable and carries favourable prognosis with 5 year survival rate of 90%. Intramucosal carcinomas have low nodal metastases risk of 5% with 90 to 100% 5yr survival rate(38). Lymph node metastases increases to 45% if submucosa is involved and the survival rate falls from 85% to 45% with nodal involvement. Treatment options are EMR(Endoscopic Mucosal Resection)(39) and ablative procedures including photodynamic therapy are providing curative results.(40)

- Variants of SCC

  Sarcomatoid carcinoma

  Basaloid SCC

  Verrucous SCC

1. Sarcomatoid carcinoma

Has biphasic microscopic features, having both carcinomatous and spindle cell components (41). Current concept is, the spindle cell component though mesenchymal in nature is derived from epithelial origin due to permanent change in EMT pathway leading to monoclonal tumors, which originate by sarcomatous metaplasia of malignant epithelial cells(42). Most common location is middle or lower third of esophagus presents usually as bulky exophytic masses measuring approximately 6cms in dimension. Microscopically, both sarcomatous and carcinomatous elements appear, former forms the bulk of tumor having stellate or spindle shaped cells in storiform pattern. Giant cells, cartilaginous or osseous differentiation can be seen,adenocarcinomatous elements and undifferentiated carcinoma also reported.(43). It can be mistaken for primary sarcoma if carcinomatous component is not evident. Prognosis is favourable with limited mural invasion.

2. Basaloid SCC(44)

Referred to as adenoid cystic carcinoma in the past behaves in a similar manner like elsewhere in the upper aerodigestive tract.. Peak incidence is 6^{th} to 7^{th} decade with M:F ratio of 3:1.Most common location is middle third of esophagus.The specific microscopic features are invasive lobules of basaloid cells exhibiting nuclear crowding, pleomorphism, necrosis, peripheral palisading and mitoses with foci of squamous differentiation. Clinical features are similar to other squamous cell carcinoma.

3. Verrucous SCC

Peak incidence – 5^{th} to 6^{th} decade with male predominance of 4:1. Most common site is middle third of esophagus. It resembles verrucous carcinoma of other sites in upper aerodigestive tract. Grossly presents as large exophytic mases with papillary fronds covered by well differentiated sqamous epithelium. With bland cytologiacal features and mild nuclear atypia, with varying degrees of parakeratosis and hyperkeratosis,diagnosis is easily missed in small biopsies unless attention is given to size, circumscription and presence of necrosis in endoscopy.(45). These tumors have pushing mode of invasion. Clinically these tumors behaves as slow growing and locally aggressive with fistulous tract formation.(46)

4. Adenocarcinoma

30 to 40% of esophageal carcinomas are adenocarcinomas(47). In majority of cases, it arises from Barrett esophagus.Peak incidence is around sixth to seventh decade. Persons with Barret's esophagus posses annual risk of 0.4% of adenocarcinoma.(48). Predisposing factors are heavy tobacco abuse, alcohol abuse and increased BMI..

Pathologic features

Most common site is lower third of esophagus. Grossly presents as irregular, flat plaques or depressed lesions or ulcerating, fungating masses. Superficial adenocarcinomas may not be evident grossly.(49). Microscopically

the histological patterns are similar to gastric adenocarcinomas, Usually Barrett's esophagus is present adjacent to the tumor. Gland formation and mucin production are evident often with intestinal metaplasia and less commonly diffuse infiltrative signet type of cells were seen.

- **Molecular genetics (11), (12).**

SCC of esophagus occurs through somatic mutations, epigenetic changes in TSG and cell adhesion molecules. Mutations/ over expressions of P53present in majority of the cases of SCC. In microspectrophotometric determination, it was found that poorly differentiated tumors harbours high DNA ploidy. Aneuploid pattern is seen in DNA studies of 75% 0f cases and correlates with higher incidence of nodal metestases and higher grade.

- **Prognosis of esophageal malignancies**

The overall prognosis is very poor with a median survival of < 1year for esophageal malignancies. Females have better prognosis compared to males. Intramucosal carcinomas and superficial carcinomas were potentially curable. bad prognosis is associated with, > 2 lymph nodes involvement, overexpression of EGFR(Epidermal growth factor receptor) and P53.

GASTRIC TUMORS

WHO classification(50)

I.Epithelial tumors

-Intraepithelial neoplasia—Adenoma

Carcinoma

1. Adenocarcinoma

- Intestinal type

- Diffuse type

    a. Papillary adenocarcinoma

    b.Tubular adenocarcinoma

    c. Mucinous adenocarcinoma

    d. Signet-ring-cell carcinoma

2. Adenosquamous carcinoma
3. Squamous cell carcinoma
4. Small cell carcinoma
5. Undifferentiated carcinoma
6. Carcinoid (well-differentiated endocrine neoplasm)

II.Nonepithelial tumors

Benign	Malignant
Leiomyoma	Leiomyosarcoma
Schwannoma	Kaposi's sarcoma
Granular cell tumor	Lymphomas Marginal zone B-cell lymphoma of MALT type Mantle cell lymphoma Diffuse large B-cell lymphoma
Glomus tumor	

Gastrointestinal stromal tumor

-Benign

-Uncertain malignant potential

-Malignant

Secondary tumors/metastatic tumors

Gastric carcinomas

Incidence is more common in Japan, Chile and Italy (51) and decreasing in countries like England and United States. All gastric malignancies were thought to arise from stem /basal cells of foveolae(52). Gastric carcinomas are mostly associated with chronic atrophic gastritis (associated with H.Pylori infection) and preceded by stages of dysplasia, in situ carcinomas and superficial carcinoma.(53). In 90% cases associated with hypochlorhydria and increased intragastric pH leads to bacterial overgrowth and conversion of dietary nitrate to nitrite and leads to production of N- Nitrosocompounds which are carcinogenic (54). Other conditions associated are gastric polyps, Menetrier disease, peptic ulcer and gastric stump. EBV association is reported in some cases with male predominance(55).

- Morphology and classification of gastric carcinoma(53)

Table 1: classification of gastric carcinomas

1.Bormann, 1926.	**2.Stout, 1953**	**3.Lauren, 1965**	**4.Ming,1977**	**5.Japanese society for gastric cancer, 1981**	**6.WHO 2000**
TypeI (Polypoid)	Superficial spreading	Diffuse	Expanding	Papillary	Already enlisted
Type II (Fungating)	Fungating	Intestinal	Infiltrative	Tubular	
Type III (ulcerative)	Penetrating			Poorly differentiated	
Type IV (infiltrative).	Linitis plastica. No special type			Mucinous Signet ring	

Other types of gastric carcinomas are :

Tumors with neuroendocrine differentiation.

Small cell carcinoma.

Adenosquamous and squamous cell carcinoma.

Mucinous carcinoma.

Parietal gland or oncocytic carcinoma.

Fundic gland carcinoma.

Lymphoepithelioma like carcinoma, EBV associated (56).

Sarcomatoid carcinoma.

Carcinoma with rhabdoid features.

Micropapillary carcinoma with frequent lymph node metastases

Acinar cell carcinoma.

Clear cell (glycogen rich) carcinoma.

- Pathological features of gastric carcinomas

1. Two extremes in gross presentation of gastric carcinomas were found, one end with fungating growth growing into the lumen and other with deeply invasive, flat, ulcerative lesion extending through the wall of the stomach(57).

2. Site prelidiction of gastric tumors (58)

Anterior wall >posterior wall>lesser curvature>greater curvature.

Carcinomas present in fundic regions likely to invade beyond the submucosa compared to those present in the pylorus(59).

Mostly all belong to adenocarcinoma category with one of the following four cell types, namely intestinal columnar, goblet cell, foveolar and mucopeptic(60).

3. Mainly two categories exist (by Lauren)

Intestinal (53%) and diffuse (33%), others are unclassifiable or mixed(61).

- Intestinal type adenocarcinomas

Thought to originate from metaplastic epithelium which is supported by ancillary studies (immunohistochemical and microscopic features)(62). The tumor size is inversely related to degree of differentiation of the tumor(63). Well differentiated tumors simulate a complete type of intestinal metaplasia with most of the cells are columnar and mucin secreting(64). Poorly differentiated tumors predominantly shows solid pattern(28).

- Diffuse type adenocarcinoma

Classically known as linitis plastica, now designated as signet ring or adenocarcinoma. This type predominantly occurs in the young (65).

Gross

It presents as thickened and rigid stomach wall usually begins in the pyloric region leading to pyloric obstruction. Cut sections show submucosal fibrosis with either presence or absence of ulceration. Comb like appearance is seen due to segmentation of hypertrophied muscle by thin, parallel, grayish white longitudinal lines.

Microscopy

Diffuse pattern of growth of malignant cells seen associated with extensive fibrosis along with inflammation with involvement of the entire wall. In one type called intramucosal carcinoma, mucosal involvement is more compared to deeper layers. Gland formation is less with mostly individual tumor cell growth. Mucin production is mostly intracellular leading to typical signet ring appearance. Extracellular mucin may also be found but if signet ring appearance is present, it should be designated as signet ring carcinoma rather than mucinous carcinoma.

- Early gastric carcinoma

Defined as carcinoma involving the mucosa and submucosa and not involving the muscularis externa, regardless of the lymph node status. Divided further into minute (<5mm) and small (6 to 10mm) in greatest dimensions(51). Endoscopic variants are type 1 or protruding, type2 or superficial, type 3 or excavated(66). Most commom site is distal third of stomach > cardia. Nodal

metastases is 5% in intramucosal tumors and risk increases to 20% if submucosal involvement is present(53).

- **Spread and metastases of gastric carcinoma**

Tumors of proximal stomach invades to esophagus, while distal stomach involves duodenum. Hepatoduodenal nodes are involved in tumors of distal third of stomach. Serosal spread is common with tumors of infiltrating type. Local extension to spleen, colon, pancreas and omentum can occur.Tumor can spread to perigastric, periaortic and celiac nodes after invading rich mucosal and submucosal Bormann's lymphatic plexus(67).

Frequency of metastases

Liver>peritoneum>lung>adrenal gland and ovary

Krukenberg's tumor is bilateral ovarian metastases from primary diffuse type of gastric carcinoma.

- **Molecular genetics (13)**

Different pathways exists for intestinal and diffuse type of gastric carcinomas. Multistep progression from gastritis to intestinal metaplasia, dysplasia and carcinoma where as the latter arises denovo.

Gene expression profiling studies found that the diffuse type involves altered expression of genes related to cell – matrix interactions and

extracellular matrix components while the intestinal type involves genes related to enhancement of cell growth(14).

In families with hereditary diffuse gastric cancer, mutations of CDH 1 gene (truncating germline mutations of E- cadherin) were found. Prophylactic gastrectomy is advised for these patients because of high penetrant risk and usually occult intramucosal type of signet ring carcinoma is found in these patients undergoing gastrectomies. Females harbouring these mutation are at risk of developing lobular carcinoma of the breast.(15).

So it is clearly understood that either loss / decreased expression of E – Cadherin, which is a componenent of cell adhesion leads to dyscohesive manner of growth resulting in diffuse type of gastric carcinomas.(16).

-In intestinal type of gastric carcinomas

Microsatellite instability (MSI) is found in upto 50% of the cases(17) and associated with frame shift mutations of TGFβRII, IGFIIR, BAX, MSH 6, MSH 3 and E2F4.(18). Also express TP53 mutations and high hTERT expression and some cases show strong membrane positivity for HER2 /c-EBRB -2 due to amplification of the HER 2 gene. This later feature helps in targeted treatment with trastuzumab(19).

Hypermethylation of the RUNX3, tumor suppressor gene is also implicated in 60% of gastric carcinomas, mostly of intestinal type.

APC gene mutations contribute for 20% of gastric adenocarcinomas in contrast to >75% in adenomas or flat dysplasias. It is clearly evident from this that APC gene mutations will not play a significant role in progression of adenoma to carcinoma.(20).

- **Prognostic factors**

Overall prognosis of gastric carcinoma is poor with low 5 yr survival rate of 15%. The following are adverse prognostic factors :(68)

1. Age > 70years.
2. Tumor location (proximal worser than distal)
3. Lymphovascular invasion.
4. CEA levels >10ng/ml
5. CA19 9 levels >37 μg/ml

In histopathological variants, intestinal type has better survival rates because of its earlier presentation and less advanced disease. When stage for stage matching is done between the tumor types, no difference in survival has been noted(69).

SMALL INTESTINAL CARCINOMAS

Annually 6000 new cases are detected in the United States per year. The median age of presentation being 55 to 67 years(61).Inspite of large surface area, incidence is quite low in small intestinal carcinomas compared to large intestine, the reasons discussed before.

WHO Histologic Classification of Tumors of the Small Intestine

1. Epithelial tumors

Adenoma

-Tubular

-Villous

-Tubulovillous

Intraepithelial neoplasia (dysplasia) associated with chronic inflammatory diseases

-Low-grade glandular intraepithelial neoplasia

-High-grade glandular intraepithelial neoplasia

Carcinoma

- Adenocarcinoma

-Mucinous carcinoma

-Signet-ring cell carcinoma

-Small cell carcinoma

-Squamous cell carcinoma

-Adenosquamous carcinoma

- Undifferentiated carcinoma

Carcinoid (well-differentiated endocrine neoplasm)

- Gastrin cell tumor, functioning (gastrinoma) or nonfunctioning

-Somatostatin cell tumor

-EC cell, serotonin-producing neoplasm

-L-cell, glucagonlike peptide and PP/PYY-producing tumor

-Mixed carcinoid—adenocarcinoma

-Gangliocytic paraganglioma

2. Nonepithelial tumors

Benign	Malignant
Lipoma	Leiomyosarcoma
Leiomyoma	Angiosarcoma
	Kaposi sarcoma

Gastrointestinal stromal tumor

Others

3. Malignant lymphomas

Immunoproliferative small intestinal disease (includes α-heavy chain disease)

Western type B-cell lymphoma of MALT

Mantle cell lymphoma

Diffuse large B-cell lymphoma

Burkitt lymphoma

Burkittlike/atypical Burkitt lymphoma

T-cell lymphoma

Enteropathy associated

Unspecified

Others

Secondary tumors/metastatic tumors.

Polyps of small intestine

-Hyperplastic polyp (metaplastic)

-Peutz-Jeghers polyp

-Juvenile polyp

- **Small intestinal carcinomas**

Most common type of small intestine carcinoma is adenocarcinoma, constituting 40% of malignancies of small intestine. Distribution is more in upper portion of small intestine compared to distal portion, 48% in duodenum, 32% in jejunum and 19% in ileum(70). Compared to large intestine, small intestinal adenocarcinomas are 40 to 60 times less and no sex prelidiction is seen in the latter(71).

Associated with many syndromes (28)

-Familial Adenomatous Polyposis

-Hereditary Nonpolyposis Colorectal Carcinoma (Lynch) syndrome

-Peutz–Jeghers syndrome

- Recklinghausen disease.

Brush cytological diagnosis is ameneable for duodenal carcinomas due to its papillary configuration(72).

- Gross features:

The small intestinal carcinomas presents as stenosing, ulcerative, flat, infiltrative or polypoidal lesions. Distally located tumors presents as napkin ring like appearance, leading to stenosis and dilatation of proximal bowel(73). Most of the tumors arise from preexisting adenomas, frequently reported as adenoma with dysplasia in endoscopic biopsies.

- Microscopy

Small intestinal carcinomas develop along the same adenoma (dysplasia) carcinoma sequence as seen in the colon. Therefore, the lesions begin as adenomas (or as areas of dysplasia in Crohn disease) that progressively increase in size and eventually develop into metastasizing carcinomas. The rich lymphatic system located superficially in small intestine makes it to be termed as carcinoma even if lamina propria is invaded by neoplastic cells in contrast to tumors involving the colon, where invasion of submucosa should be present to designate it as carcinoma due to the deep location of lymphatic plexus in the latter.

Other types of carcinomas are

-Small cell (neuroendocrine) tumors.

-Adenosquamous carcinoma.

-Anaplastic (sarcomatoid) carcinoma.

LARGE INTESTINAL CARCINOMAS

Prevalence of large intestinal carcinomas are high in north west Europe and North America but low in Asian, African countries and South America. These are the most curable forms of cancers of the GIT. Increased incidence among young blacks is noted. Mean age of presentation is 6^{th} decade with equal sex prelidiction. Cancers occuring in young age (<40 yrs) were mostly located in distal colon and rectum with aggressive behavior or having one of hereditary colorectal cancer syndromes(74). Elderly patients mostly presents with medullary type of cancers.

- Syndromes associated with colorectal cancers

-Familial Adenomatosis Polyposes (FAP).

-Hereditary nonpolyposis colorectal cancer syndrome (HNPCC) / Lynch syndrome.

-Torre – Muir syndrome.

-Familial colorectal carcinoma

WHO Histologic Classification of Tumors of the Colon and Rectum

1.Epithelial tumors

Adenoma

-Tubular

- Villous

-Tubulovillous

-Serrated

Intraepithelial neoplasia (dysplasia) associated with chronic inflammatory diseases

-Low-grade glandular intraepithelial neoplasia

- High-grade glandular intraepithelial neoplasia

Carcinoma

-Adenocarcinoma

-Mucinous carcinoma

- Signet-ring cell carcinoma

- Small cell carcinoma

- Squamous cell carcinoma

- Adenosquamous carcinoma

-Medullary carcinoma

-Undifferentiated carcinoma

Carcinoid (well-differentiated endocrine neoplasm)

- EC cell, serotonin-producing neoplasm

-L cell, glucagon like peptide and PP/PYY-producing tumor

-Others

Mixed carcinoid—adenocarcinoma

2.Nonepithelial tumors

Benign Malignant

Lipoma -Leiomyosarcoma

Leiomyoma -Angiosarcoma

- Kaposi sarcoma -Malignant melanoma

Malignant lymphoma

-Marginal zone B-cell lymphoma of MALT type

- Mantle cell lymphoma

- Diffuse large B-cell lymphoma

- Burkitt lymphoma

- Burkitt like/atypical Burkitt lymphoma

- Others

Secondary tumors

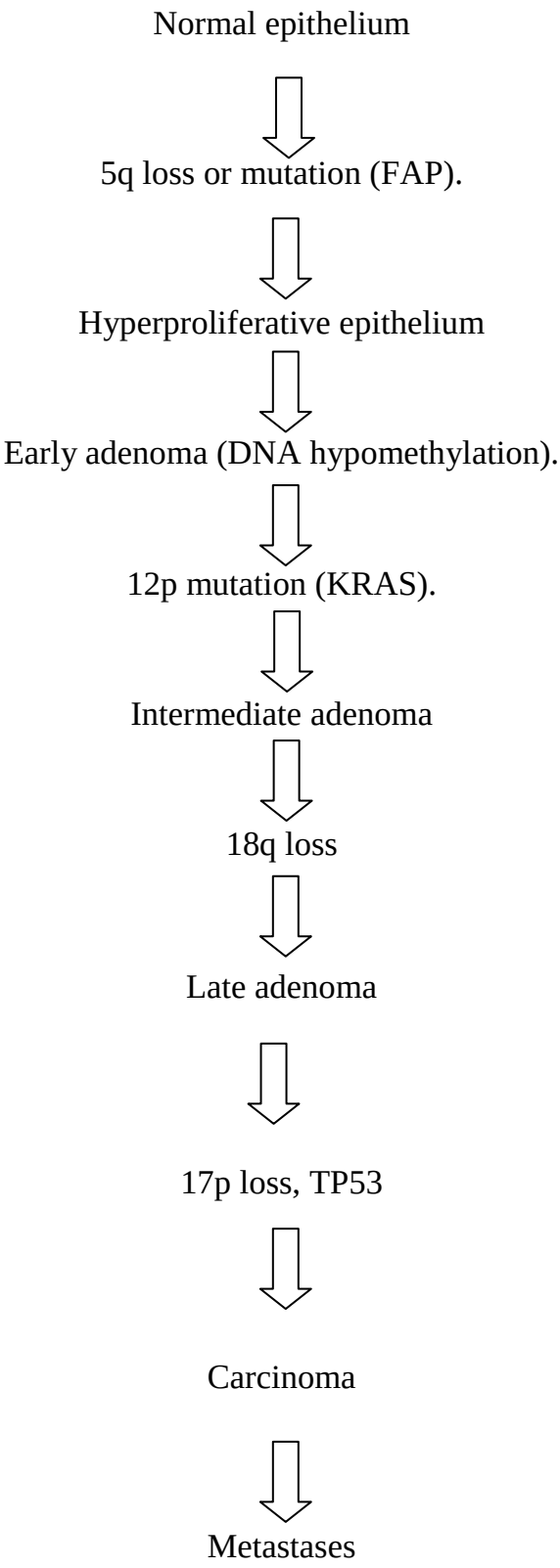
Polyps

- Hyperplastic polyp (metaplastic)

-Peutz—Jeghers

-Juvenile

Figure 5 : schematic representation of Adenoma carcinoma sequence



Gross features of colorectal carcinomas

They present as either polypoidal or ulcerative / infiltrative. The polypoidal type presents as bulky mass with sharp delineation from the normal bowel, where as the latter one as elevated margins with central ulceration. In Japanese literature one variant of the latter was termed as flat/depressed carcinoma having significant lympho vascular invasion compared to polypoidal type(75). These type of carcinomas usually arise denovo rather than malignant transformation from an adenoma.

- Microscopic variants

Usual type is well to moderately differentiated adenocarcinoma producing mucin,usually elicits an inflammatory response at the edge of the tumor.with production of mainly T lymphocytes and eosinophils. The tumor may invade through all layers of the bowel involving pericolic fat, perineural spaces and veins. Metaplastic bone formation may be seen rarely in the tumor stroma.

Other variants

1. Mucinous carcinoma(76),(77)

Comprises around 15% of colorectal malignancies and usually presents at v younger age group. In this type, large lakes of extracellular mucin seen admixed with tumor cells. Atleast 50% of tumor should comprise mucinous

foci to designate this type. Usually presents at an advanced stage with poor prognosis, than conventional adenocarcinoma .

2. Signet ring carcinoma(linitis plastica type)(53)

Rare type associated with extremely poor prognosis. Microscopically the accumulation of intracellular mucin pushes the nucleus to one side giving the appearance. Primary from the breast and ovary should be ruled out before diagnosing primary signet ring carcinoma in the colon.

3. Serrated adenocarcinoma

4. Basaloid (cloacogenic) carcinoma

5. Clear cell carcinoma

6. Hepatoid adenocarcinoma

7. Medullary (solid, undifferentiated) adenocarcinoma

8. Anaplastic (spindle and giant cell, sarcomatoid) carcinoma

- **Staging and grading of colorectal carcinomas**:

1. Dukes system (1937) is directly related with prognosis.

Stage A – Involving only the wall of the bowel.

Stage B – Extending through the wall.

Stage C - Lymph node metastases.

Subdivided into C 1- regional nodes involved.

C2 - nodes at mesenteric blood vessel ligature.

Stage D - Distant metastases.

2. Astler and Coller system (1954)

Stage A – Tumor limited to mucosa.

Stage B1 – Involving upto muscularis externa (but not penetrating).

Stage B2 – Penetrating muscularis externa.

Stage C1- Limited to bowel wall but with nodal metastases.

Stage C2 – Extending through bowel wall with nodal metastases.

Other systems

3. AJCC System (American Joint Committee on Cancer).

Jass et al (78) – considering peritumoral lymphocytic infitration and tumor growth pattern as indicators for prognosis.

- Salient clinical features of colorectal cancers

1. Altered bowel habits (alternating constipation and diarrhea).

2. Anemia (chronic blood loss).

3. Left sided colon tumors presents with intestinal obstruction.

4. 25% of caecal tumors presents with signs of acute appendicitis.

- **Spread and metastases of colorectal carcinomas:**

Most common sites are lymph nodes and liver.

Lymph node metastases occurs in poorly differentiated tumors and tumors with highly infiltrative patterns. Average number of nodes to be recovered from colorectal carcinoma specimen as recommended by some authors is around 15 nodes (79).

At National cancer institute of Merlin, by meticulous manual dissection they found out an average of 36 nodes per patient in a consecutive study of 50 cases of rectal carcinomas.

Pericolonic tumor deposits (PTD's) should be distinguished from lymph node metastases. PTD's may be present in perineural, perivascular or intravascular location beyond the muscularis propria (80). These may have some impact in TNM staging of colorectal carcinomas.

Tumors showing blood vessel invasion are more prone for liver metastases. As ovaries were also involved in high proportion of cases, bilateral oophorectomy is performed during initial resection of the tumor as a prophylactic measure. At many other sites metastases from the colorectal carcinoma mimicks primary adenocarcinoma of involved site, for example in ovarian metastases, it appears as primary endometrioid (secretory variant) or

clear cell variant(81). In prostrate, it appears as primary adenocarcinoma of the prostrate.

Prognosis of colorectal malignancies(82)**,**(83)

- Age :

Two extremes of age, very young and very old age have poor prognosis. Age plays an important role in rectal than colon carcinomas.

- Sex:

Females have better outcome compared to males.

- Serum CEA levels:

Levels > 5ng/ml associated with bad prognosis. It is considered as an independent prognostic factor.

- Tumor location:

Left sided tumors will have good prognosis compared to right sided tumors. Tumors arising from sigmoid colon and rectum will have bad prognosis.

In one study, they found out that left sided tumors were presenting with late recurrances.

- Tumor edge:

Tumors with polypoidal edge will have better prognosis compared to one with non-polypoidal edge.

- Tumor margins and inflammatory reaction

The presence of inflammatory infiltrates at the tumor interface and presence of degenerative changes within the tumor lead to beter prognosis compared to those who lack these features.

- Microscopic type:

Medullary type will have good prognosis. Signet ring, anaplastic and mucinous type will have bad prognosis.

Other bad progostc indicators are

1. Tumor angiogenesis
2. Mucin related antigens
3. KRAS mutation
4. Perineural invasion
5. Pericolonic tumor deposits

- **Molecular genetics(21)**

According to Fearon and Vogelstein, MMR gene is inactivation is caused by either somatic mutations or by epigenetic inactivation (MLH 1). The mutational inactivation of MMR genes occurs as second hit, who already harbours germline mutation. Tumors associated with MSI are poorly differentiated or mucinous with prominent host response, circumscribed growth pattern and right sided lesions. Tumor infiltrating lymphocytes are best predictors of MSI.

Oncogenes involved in somatic mutations were KRAS, BRAF,PIK3 and CTNNB1(β catenin). In 40% of cases mutations of the RAS is found.

Tumor suppressor genes involved are TP53,APC,DPC4/SMAD4,DCC and MCC. Of these majority of the cases show mutations of TP53.

ANAL CARCINOMAS

More common in middle age group, with female predominance of 5:1. Anal carcinomas are highly associated with HPV infection, particularly HPV 16, detected in 82% of the cases.

WHO Histologic Classification of Tumors of the Anal Canal

1. Epithelial tumors

Intraepithelial neoplasia (dysplasia)

Squamous or transitional epithelium

- Glandular

-Paget disease

Carcinoma

-Squamous cell carcinoma

-Adenocarcinoma

-Mucinous adenocarcinoma

-Small cell carcinoma

-Undifferentiated carcinoma

Carcinoid tumor

2. Malignant melanoma

3. Nonepithelial tumors

4. Secondary tumors

Most of the anal carcinomas are underdiagnosed and eventually leading to advanced stage of presentation. Most common clinical presentation is bleeding followed by pain. The site of origin of anal carcinomas are divided into

Upper (colorectal) zone – adeno carcinoma, small cell carcinoma and mucinous adenocarcinoma.

Middle, Anal Transformation Zone (ATZ) – squamous, basaloid, melanoma and mucinous adenocarcinoma.

Lowest (squamous) zone - squamous cell carcinoma and melanoma.

- Gross features

Anal carcinomas usually arise from the pectinate line and grows either upward into the rectum and surrounding tissues or outward to the perianal tissues.

Involvement of the perianal skin may be superficial, with only surface ulceration and slightly elevated margins.

- Microscopic features

(1) Squamous cell carcinoma

(2) Cloacogenic (transitional, basaloid)

Dougherty and Evans (51)have proposed to subdivide them into five types: keratinizing, nonkeratinizing, basaloid, with mucous cysts, and pseudoadenoid cystic. The last should be distinguished from true adenoid cystic carcinoma of salivary gland type, from which it differs at the immunohistochemical level.

- **Paget's disease of the anal canal** (84)

Classified into following two types

1. Cutaneous type (apocrine).

2. Endodermal type (adenocarcinoma like)

Apocrine type is purely intraepithelial where as endodermal type is associated with invasive rectal adenocarcinoma.

Prognosis of Paget's disease depends on presence or absence of invasive component and involvement of inguinal node.

- **Malignant melanoma of the anal canal:**(85), (86).

The ratio of melanoma: SCC: Adenocarcinoma is 1:8:250 and most common in adults. Mostly arises from the pectinate line and grows towards the rectal ampulla. Most common symptom is rectal bleeding > mass>pain.

Grossly it present as polypoidal mass either single or multiple with smooth surface and it mimicks thrombosed hemorrhoids in early stage.

Microscopically, the tumor is usually pigmented with junctional component and also lentigenous appearance in adjacent mucosa similar to melanomas in other sites.

Prognosis is extremely poor and is related to tumour size and depth of invasion.

Even, rare adenocarcinomas arising in this region will have plenty of melanin accumulation in tumor cells due to colonization mechanism as seen in breast carcinoma and it should be differentiated from malignant melanoma.

Prognosis of anal carcinomas

Bad prognostic factors

1. Older age
2. Proximally located tumors
3. Tumor stage
4. Inguinal node involvement
5. Microscopic grade
6. Basaloid type

- **Molecular genetics(22)**

P53 overexpression is sen in >85% of the cases but TP53 mutation is rare. High SOX 2 and low CDX2 expression is seen in anal carcinomas.

Loss of heterozygosity at 11q - is early event in anal carcinogenesis.

Additional molecular events leading to invasive carcinoma are allelic loss at 5q (APC), 17p(TP53), and 18q (DCC). In HIV patients, loss of 11q heterozygosity is more common.

Aberrant DNA methylation is present in most cases of AIN 2 and AIN 3, but not in normal mucosa and AIN 1.

IMMUNOHISTOCHEMICAL FEATURES OF GASTROINTESTINAL TRACT MALIGNANCIES(91)

Immunohistochemistry Principle

Immunohistochemistry (IHC) is a method for detecting antigens or haptens in cells of a tissue section by exploiting the principle of antibodies binding specifically to antigens in biological tissues. The antibody-antigen binding can be visualized in different manners. Enzymes, such as Horseradish Peroxidase (HRP) or Alkaline Phosphatase (AP), arecommonly used to catalyze a color-producing reaction.

- Major components in a complete immunohistochemistry experiment:

1) Primary antibody binds to specific antigen;

2) The antibody-antigen complex is formed by incubation with a secondary, enzyme- conjugated, antibody;

3) With presence of substrate and chromogen, the enzyme catalyzes to generate colored deposits at the sites of antibody-antigen binding.

- **IHC features of Esophageal carcinomas:**

Immunohistochemical profile of SCC of esophagus is similar to that of its skin counterpart: CK7-, CD20-, CK5/6+, CK10+ and CK14+.SCC is always positive for p63. Additionally, most cases of esophageal SCC are also positive for p53, a finding not seen in normal esophageal mucosa.

Most cases of esophageal adenocarcinoma involve the lower one third of the esophagus and show glandular differentiation. These tumors usually express CK7, variable CK20, AMACR, and weak focal CDX-2, an immunohistochemical pattern similar to that of gastric adenocarcinoma.P16 is negative in esophageal adenocarcinoma unlike SCC (26)

- **IHC features of Gastric carcinomas**

Majority of gastric adenocarcinomas is located in the pylorus and antrum (50-60%), followed by the cardia (25%), and the body or fundus (15-25%) and may be exophytic, flat or ulcerated.

Table 2. shows Comparison of immunohistochemical expression of various IHC markers between adenocarcinomas of esophagus & stomach and Barrett's esophagus.

Immunohistoche-mical markers	Barrett's esophagus		Esophageal adenocarcinoma	Gastric adenocarcinoma
	without dysplasia	With dysplasia		
AMACR	0%	98%	73%	/
HepPar-1		97%	13%	31%
Keratin 7		97%	94%	51%
Keratin 20		95%	45%	48%
MUC1	40%	23%	47%	31%
MUC2	95%	95%	0%	29%
CDX-2	77%	37%	46%	60%
P53	0%	75%	57%	/
Ki-67	11%	85%	76%	/

Intestinal type GA shows variable expression of CK7, CK20 CDX-2, MUC1, and MUC5AC (45-47). Diffuse type of GA usually develops de novo, and is not associated with H. pylori induced IM. Over 70% of cases of the

diffuse type of GA are positive for CDX-2, CK, HepPar-1 and variable expression of, MUC2 and MUC5AC, but negative for MUC1 and E-cadherin ((48,49). Cases of poorly differentiated adenocarcinoma with prominent lymphoplasmacytic stroma may also be positive for EBV(50,51)

- **IHC features of Small intestine carcinomas**

Small intestinal adenocarcinomas are CK7+ in more than half of all cases, unlike normal small intestinal mucosa which is CK7-

Adenocarcinomas of the small bowel are also positive for CK20, CDX-2, and villin.

- **IHC features of Colorectal cancer (CRC)**

CA is characterized by a CK7 negative and CK20 postive immunophenotype, and thus can be differentiated from non-ampullary small intestinal adenocarcinomas by their lack of expression of CK7 and positivity for CK20,

CDX-2 is a transcription factor involved in the proliferation and differentiation of intestinal epithelial cells, and the incidence of CDX-2 expression in adenocarcinoma of the gastrointestinal tract increases from esophagus to rectum and in cases where it is positive all tumor cells show a strong staining pattern.

► **IHC features of Anal tumors**

Paget disease of the anal canal may arise from an underlying anal gland adenocarcinoma, adnexal (eccrine gland) adenocarcinoma or an underlying visceral malignancy, most commonly acolorectal adenocarcinoma. The use of immunohistochemistry can help differentiate these as those arising from anal gland adenocarcinoma would be CK7+/CK20+/CDX-2+/GCDFP-15- from adnexal adenocarcinoma would be CK7+/CK20-/CDX-2-/GCDFP-15+ and that from a colorectal adenocarcinoma would be CK7-/CK20+/CDX-2+/ GCDFP-15+-

Table 3 : Expression of various Immunohistochemical markers in gastrointestinal tract malignancies

Tumor types	Keratin 7	CK20	MUC1	MUC2	MUC5AC
Esophageal adenocarcinoma	92%	62%	36%	19%	42%
Gastric adenocarcinoma, intestinal	63%	32%	0%	27%	53%
Gastric adenocarcinoma, diffuse	75%	42%	33%	11%	53%
Small intestinal adenocarcinoma*	92%	59%	52%	43%	30%
Colorectal adenocarcinoma	9%	85%	35%	40%	6%
Appendiceal adenocarcinoma	28%	100%	17%	100%	67%
Pancreatic ductal adenocarcinoma	96%	40%	87%	9%	70%
Cholangiocarcinoma	91%	35%	60%	10%	42%
Adenocarcinoma of Ampulla of Vater	58%	44%	58%	21%	28%

The immunohistochemical markers p63 and calponin reactivity will be assessed by determining the presence and intensity of staining.(90), (91).

- P 63: (staining pattern- nuclear) (90).

p63 is a member of the p53 gene family. The gene is located on chromosome 3q27-29. It encodes at least six different transcripts with transactivation (TAp63) or negative effects (DNp63) on the p53 reporter genes, resulting in tumour suppressor and oncogenic effects respectively. The p63 isoforms lack the N-terminal transactivation domain and inhibit p53. In normal tissues, expression was restricted to epithelial cells of stratified epithelia, such as skin, esophagus, ectocervix, tonsil, and bladder, and to certain subpopulations of basal cells in glandular structures of prostate and breast, as well as in bronchi[4]. In the respiratory tract, normal ciliated and goblet cells were p63 negative, but reserve cells were p63 positive. In keratinocytes, the expressed isoform is ΔNp63, which presumably maintains epithelial cell proliferation

In response to DNA damage, the expression of wild type p63 protein is upregulated and exerts an important role in maintaining the genomic DNA integrity by preventing DNA replication, allowing the cell to activate the DNA repair machinery. If there is a failure of DNA repair mechanism then p63 protein induces the cell to undergo apoptosis.

If both the copies of p63 protein gene are mutated then the tumor suppressor gene p63 lose control over cell replication and DNA repair leading to uncontrolled cell proliferation and malignancy. p63 show strong nuclear positivity in poorly differentiated carcinomas.

- **Calponin (**Staining Pattern: Cytoplasmic)(91)

Calponin is a smooth muscle-specific protein **(actin binding protein)** which binds strongly to actin in a calcium-independent manner and it stabilizes the actin filament system. The calponin binds tropomyosin and F-actin and is thought to be involved in the regulation of smooth muscle contraction. The expression of calponin is restricted to smooth muscle cells and is a marker of the differentiated contractile phenotype of developing smooth muscle. Two isoforms of calponin exist whose molecular weights are 34kD and 29kD. Expression of the 29kD form is primarily restricted to muscle of the urogenital tract. The 34kD isoform is expressed in vascular and visceral smooth muscle of trachea, jejunum and oesophagus. Immunohistochemical expression is seen in parenchymal and vascular smooth muscle. In knock-out experiments, CN deficient mice exhibited morphological fragility in tissues, such as blood vessels and uterus and peritoneal membranes leading to highly hematogenous metasta-sis and peritoneal dissemination of cancer cells. Peritoneal disease was later suppressed by treatment with CN, 8whichimplies an importance of CN to protect against cancer dissemination via the stabilization of peritoneal membranes.

It has recently been demonstrated that CN expression isdown-regulated in blood vessels in human malignant mela-noma, hepatocellular carcinoma (HCC) and renal cellcarcinoma. In HCC, CN expression levels were correlated with disease prognosis.

Additionally, Ramaswamy et al. highlighted CN as one of the nine down-regulated genes associated with adenocarcinoma metastasis by assessment with micro cDNA arrays. Thus, increasing lines of evidence have suggested to us that CN expression level in vessels of cancer tissue could be a useful diagnostic indicator of cancer metastasis and patient prognosis in conjunction with conventional staging procedures.

MATERIALS AND METHODS

- All cases of Gastro-intestinal tract malignancies obtained either as biopsy or resected specimens are included in the study group.
- Back ground details are collected.
- Most representative blocks are chosen.
- Three sections are taken one for hematoxylin and eosin stain and other for immunohistochemical stains p63 and Calponin

STUDY PLACE :

Pathology Department, Govt., Chengalpattu medical college, Chengalpattu.

COLLABORATING DEPARTMENT

Govt., Arignar Anna Memorial Cancer Hospital and Research Institute, (GAAMCH & RI), Kaarapettai.

STUDY DESIGN

Retrospective and prospective

PERIOD OF STUDY

3 YEARS (JUNE 2014-JUNE 2017)

SELECTION OF STUDY POPULATION

Cases selected from tumor register of Govt., Chengalpattu medical college, chengalpattu and GAAMCH & RI, kaarapettai.

NUMBER OF CASES PLANNED TO BE STUDIED = 60

INCLUSION CRITERIA

Tissue blocks of patients who are diagnosed as having Gastro-intestinal tract malignancies.

EXCLUSION CRITERIA

- Tissue blocks of known patients of Gastrointestinal tract carcinoma who underwent preoperative therapeutic Radiotherapy or Chemotherapy.
- Patients diagnosed as neuroendocrine tumors and lymphomas.
- Patients with non- carcinomatous Gastrointestinal lesions.

HISTOPATHOLOGICAL EXAMINATION

Histopathological categorization, pathological staging & grading of Gastro-intestinal carcinomas using Hematoxylin & Eosin stained section in correlation with Immunohistochemical markers p63 and Calponin:

Esophageal carcinoma – Grade 1 – 3.

Gastric carcinoma – Grade 1- 3.

Colorectal carcinoma – Grade 1 – 3.

Anal carcinoma – Grade 1 – 3.

Nodal status.

Depth of invasion.

IMMUNOHISTOCHEMISTRY

Expression of p63 and calponin in immunohistochemical stained section

p63 Expression:

POSITVE - staining of nucleus of tumor cells

- Intense Staining – (>50% of tumor cells positive) Poor prognosis.
- Weak staining – (<5% of tumor cells positive) Better prognosis.

NEGATIVE

Calponin Expression

POSITIVE - staining of stromal tumor blood vessels

-Intense staining – Better prognosis.

- Week staining – Poor prgnosis.

NEGATIVE

Immunohistochemistry Procedure

1. 4μ thick sections were cut from formalin fixed paraffin embedded tissue samples and transferred to gelatin-chrome alum coated slides.

2. The slides were incubated at 58ºC for overnight.

3. The sections were deparaffinized in xylene for 15 minutes x 2 changes.

4. Rehydrated through descending grades of alcohol as follows absolute alcohol x 2 changes 5 minutes each, 90% alcohol x 5 minutes

   Washed in distilled water 2 changes, 2 minutes each

5. Heat induced antigen retrieval was done with microwave oven at 150 degree Celsius with citrate buffer (pH 6.0) for 15 to 20 minutes.

6. Then cooled for 10 minutes.

7. Washed in distilled water 2 changes, 2 minutes each.

8. Washed in TBS for 2 minutes.

9. Endoperoxidase blocking was done by adding hydrogen peroxide on the section and kept for 5 minutes.

10. Washed in the wash buffer for 2 minutes twice.

11. Primary antibody P63 / Calponin Mouse monoclonal,(prediluted) was added and kept for 30 minutes in a moist chamber.

12. Then washed in wash buffer 2 minutes 2 times each.

13. Poly excel target binder reagent was added and kept for 15 minutes.

14. Washed in two changes of buffer 2 minutes each.

15. Poly excel HRP (Horse Radish Peroxidase) was added and incubated for 15 minutes.

16. Washed with buffer 2 minutes, 2 changes.

17. Working DAB chromogen (1ml DAB buffer + 1 drop chromogen, mix well) was added and kept for 2-5 minutes.

18. Then washed in distilled water.

19. Counter stained with hematoxylin for 30 seconds.

20. The slides were washed in running tap water for 3 minutes.

21. The slides were air dried, cleared with xylene and mounted with DPX.

STASTICAL ANALYSIS

The stastical analysis was done. The primary data was entered in MS excel sheet and analysed using SPSS 20V. The results were presented in terms of tables and graphs. The descriptive statistics frequency and percentages was calculated. The association between the categorical variables were analysed by Chi square test with 5% level of significance.

RESULTS

We had studied sixty cases of gastrointestinal carcinomas from June 2014 to June 2017 and also usefulness of Immunohistochemistry markers p63 and calponin in these cases. The cases diagnosed as neuroendocrine tumors and lymphomas were excluded from the study.

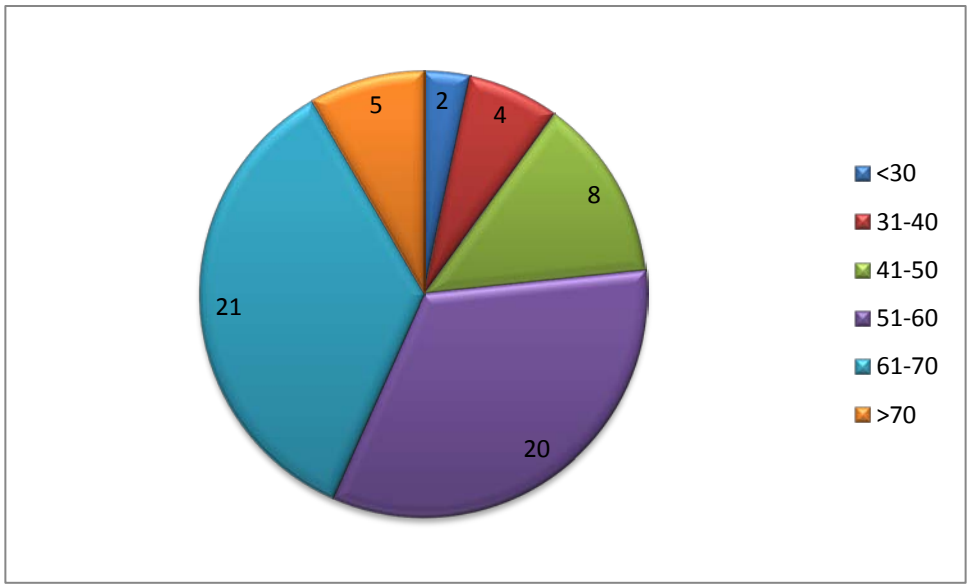
- **Age group**

Our study included age group of < 30 years of age to >70 years of age. Among these age group of patients, most of the patients are in the age group of 61 – 70 years, constituting 22 cases (35% of total cases). Only 2 cases were found in the age group of < 30 years (3.4 % of total cases).

Table 4: Age wise distribution of gastrointestinal tract carcinoms.

Age	Frequency	Percent
≤30	2	3.4
31-40	4	6.7
41-50	8	13
51-60	20	33.4
61-70	21	35
>70	5	8.5
total	60	100

Chart 1 : Age wise distribution



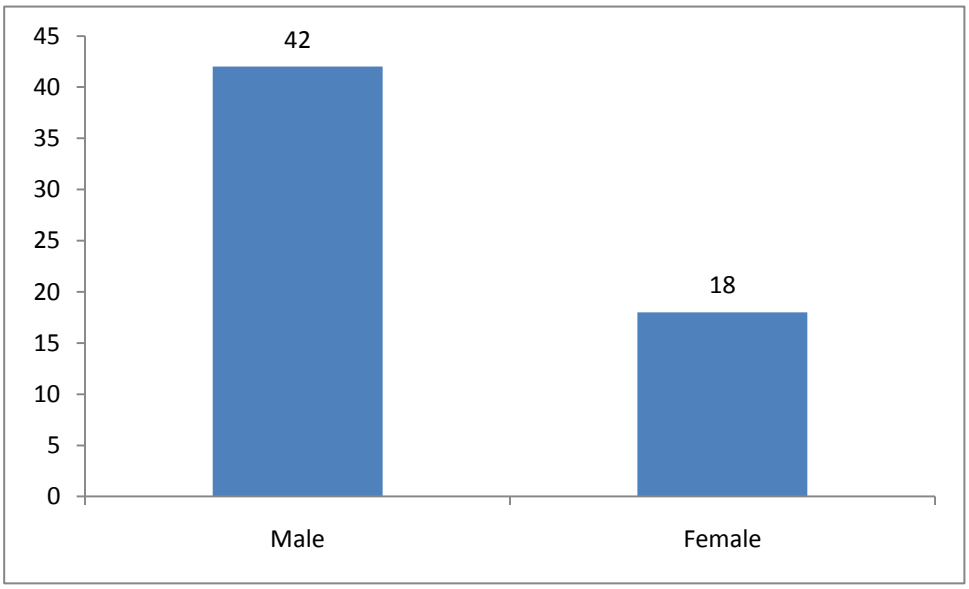
- **Gender wise distribution of the cases**

Among the sixty cases studied, 70% are male patients (42 cases) and 30% are female patients(18 cases)

Table 5: Gender wise distribution of the cases

Gender	Frequency	Percent
Male	42	70
Female	18	30
Total	60	100

Chart 2 : Gender wise distribution



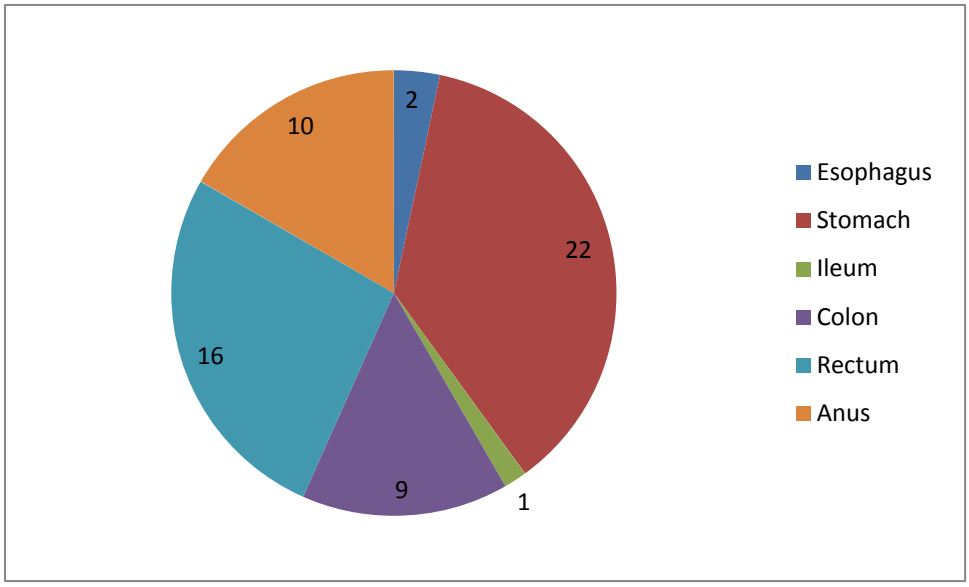
- **Anatomical site wise distribution of cases**

In our study, the most common site of gastrointestinal tract cancer is found to be stomach (antrum) constituting around 22 cases (36.7%). The second most commom site being rectum constituting around 16 cases (26.7%). Ileum was the least common site of involvement in our study.

Table 6: Anatomical site wise distribution of cases

Site	Frequency	Percentage
Esophagus	2	3.3
Stomach	22	36.7
Ileum	1	1.7
Colon	9	15
Rectum	16	26.7
Anus	10	16.7
Total	60	100

Chart 3: Anatomical site wise distribution of cases



In our study, among these cases 10% of cases (six patients) presented with gastric outlet obstruction.

- **Histopathological diagnosis wise distribution of cases**

In this study, among the sixty cases, 53 cases are adenocarcinomas (88.4%) was found to be most common histopathological diagnosis with one case of intestinal type. The least common type was found to be adenosquamous and malignant melanoma, each constituting 2 cases (3.3% each).

Table 7: Histopathological diagnosis wise distribution of cases

Histo pathological diagnosis		**Frequency**	**Percent**
Infiltrating adenocarcinoma	Conventional	47	78.4
	Intestinal type	1	1.7
	Mucinous	5	8.3
Adenosquamous carcinoma		2	3.3
Infiltrating squamous cell carcinoma		3	5.0
Malignant melanoma		2	3.3
Total		60	100

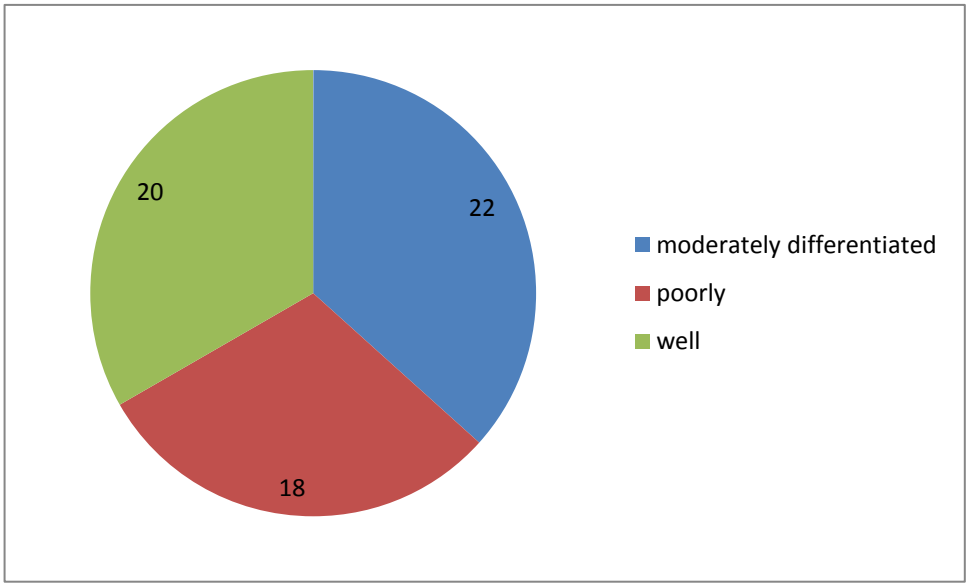
- **Histopathological grade wise distribution of cases**

Among those sixty cases, 20 cases were found to be well differentiated (28.3%). 22 cases were found to be moderately differentiated (36.7% of the total cases).18 cases were found to be poorly differentiated (33.3%).

Table 8: Histopathological grade wise distribution of cases

HPE	Frequency	Percent
Well differentiated	20	33.3
Moderately differentiated	22	36.7
Poorly differentiated	18	30.0
Total	60	100

Chart 4: Histopathological grade wise distribution of cases



IMMUNOHISTOCHEMICAL ANALYSIS AND INTERPRETATION

In our study we have analysed the role of expression of immunohistochemical markers P63 and Calponin in sixty cases of gastrointestinal tract carcinomas.Staning pattern of P63 was based on positivity of tumor cells,

Weak staining – if < 5% of tumor cells shows positivity.

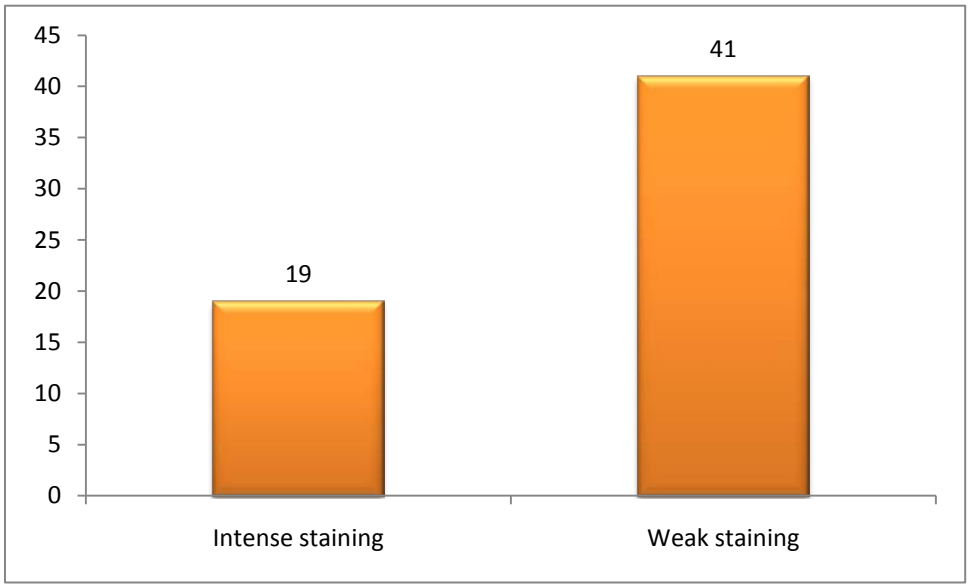
Intense staining – if > 50% of tumor cells shows positivity.

For P63, intense staining pattern is considered as significant (poor prognosis).

- **Expression of P 63**

Among sixty cases, 19 cases show intense staining and remaining 41 cases show weak staining.

Chart 5: staining pattern of P 63



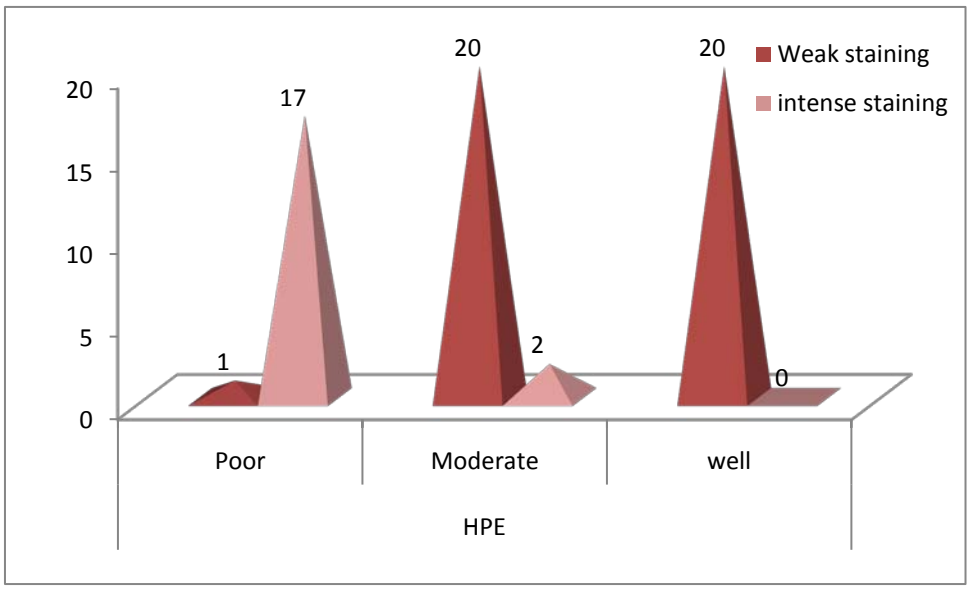
- **P63 expression in relation with histopathological grading**

Among sixty cases, intense staining of P63 was found in 17 cases of poorly differentiated carcinomas with a significant ‘P’ value of 0.001. most of the well differentiated carcinomas shows weak staining of P63.

Table 9: P63 expression in relation with histopathological grading.

P63	HPE				Chi sq	staining	p
	Poor	Moderate	well	Total		percentage	
Weak staining	1	20	20	41	47.23	68.3	0.001
Intense staining	17	2	0	19		31.7	
Total	18	22	20	60		100	

Chart 6: P63 expression in relation with histopathological grading



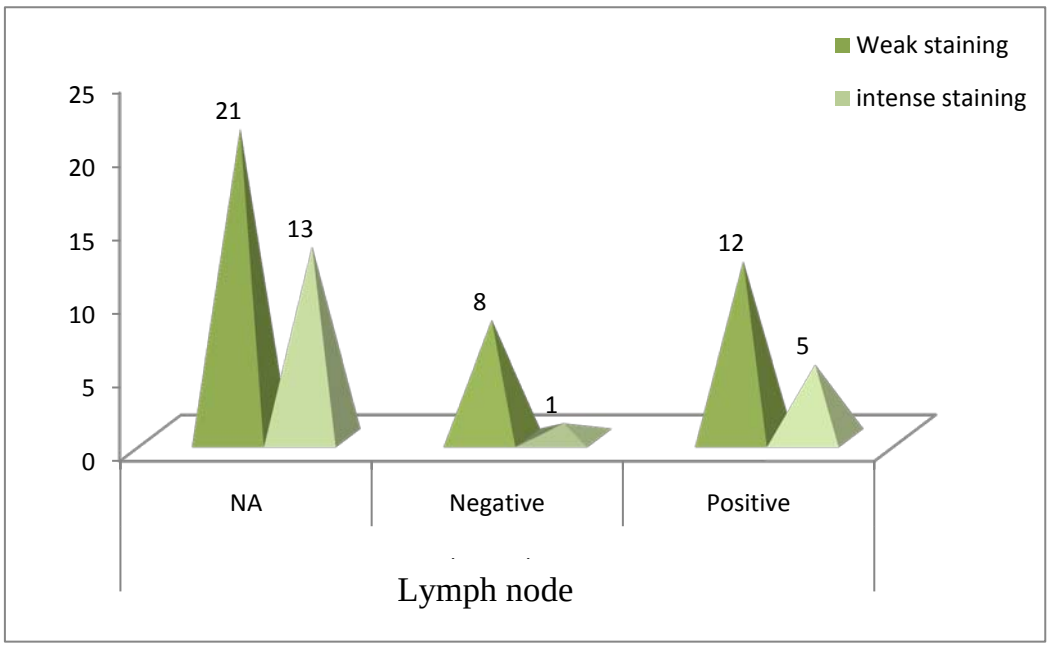
- **Comparison of P63 expression with lymph node status**

Among the sixty cases, lymph node positivity was found in 17 cases, of these 17 cases, 12 cases(with <3 positive nodes) showed weak staining of P63 and remaining 5 cases (>3 positive nodes) showed intense staining of P63. Lymph node negativity is seen in 9 cases, in these 8 cases showed weak staining of P63.

Table 10: P63 expression with lymph node status

P63	Lymph node				Chi sq	p
	NA (not available)	Negative	Positive	Total		
Weak staining	21	8	5	41	2.47	0.29
Intense staining	13	1	12	19		
Total	34	9	17	60		

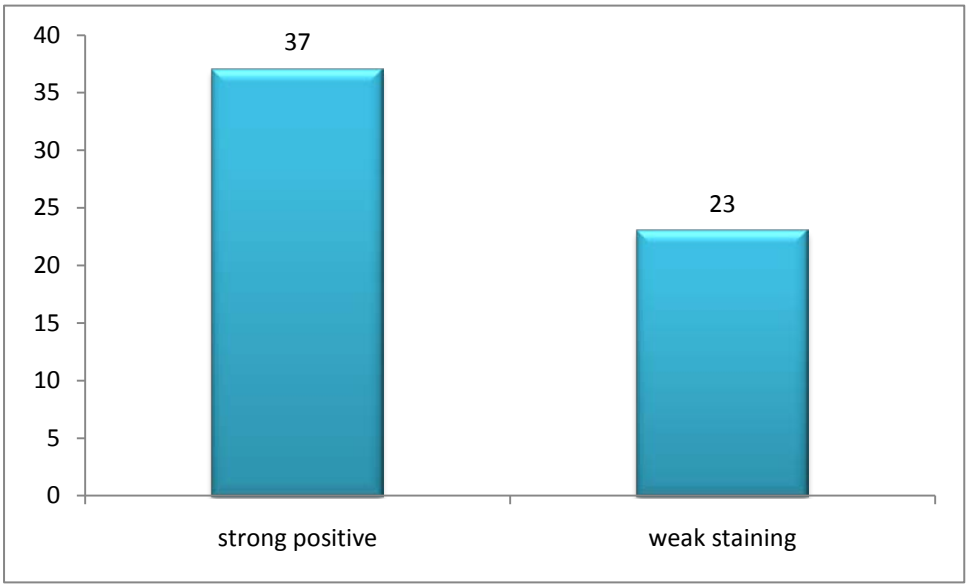
Chart 7: P63 expression with lymph node status



- **Expression of calponin**

Among sixty cases, strong positivity was seen in 37 cases (61.7%) and weak staining was seen in 23 cases (38.3%).

Chart 8: Staining pattern of Calponin



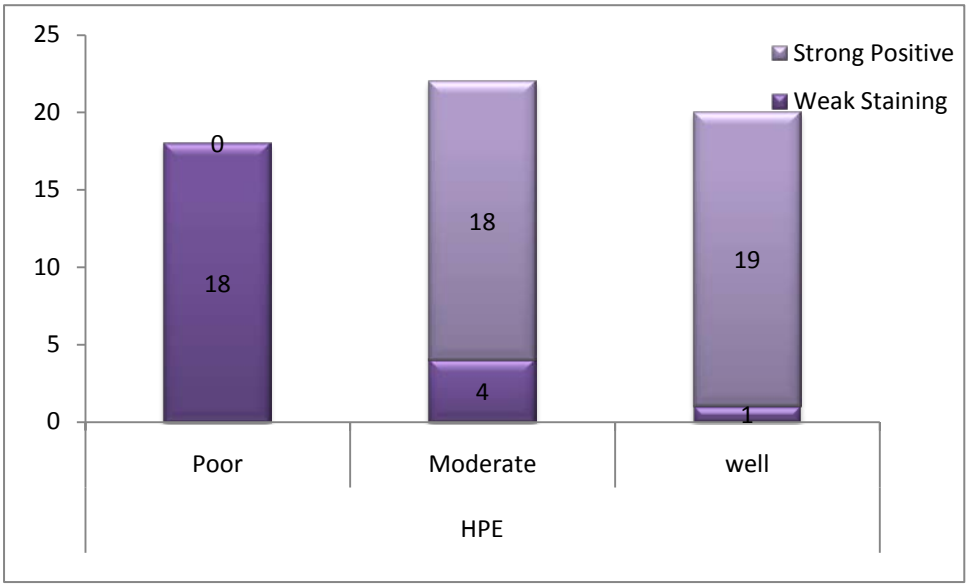
- **Calponin expression in relation with histopathologiacal grading**

Among these sixty cases, strong positivity was found in well differentiated carcinomas and weak staining was found in poorly differentiated carcinomas.

Table 11: Calponin expression in relation with histopathologiacal grading

Calponin	HPE				Chi sq	Staining	P
	Poor	Moderate	well	Total		percentage	
Weak Staining	18	4	1	23	42.14	38.3	0.001
Strong Positive	0	18	19	37		61.7	
Total	18	22	20	60		100	

Chart 9 :Calponin expression in relation with histopathologiacal grading



- **Calponin expression in relation with lymph node status.**

Among sixty cases, lymph node status was available for 26 cases. Lymph node positivity was seen in 17 cases and negative lymph node status was seen in 9 cases. Of these 17 cases, Calponin expression showed strong positivity in 9 cases and weak staining in 8 cases. In the latter group, all 9 cases showed strong positivity. ‘P’ value was found to be significant of < 0.03.

Table 12 Calponin expression in relation with lymph node status

Calponin	Lymph node				Chi sq	p
	NA	Negative	Positive	Total		
Weak Staining	15	0	8	23	6.62	0.03
Strong Positive	19	9	9	37		
Total	34	9	17	60		

Chart 10 : Calponin expression in relation with lymph node status.

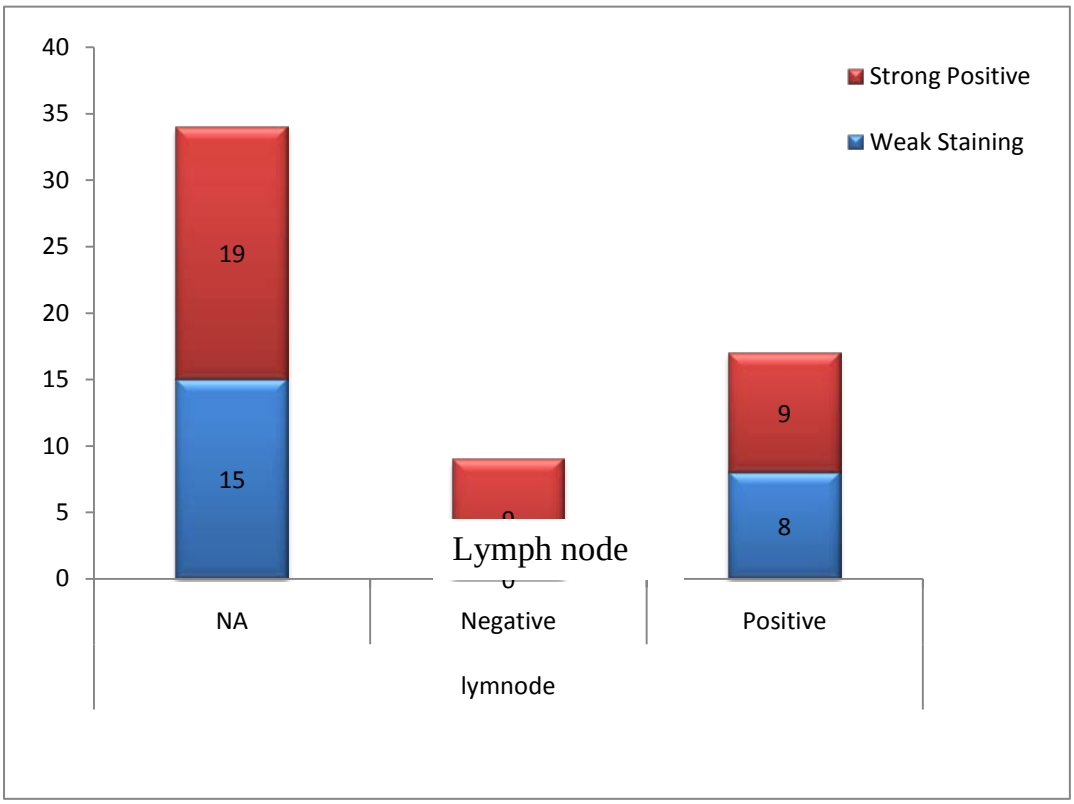


Table 13: Comparison of staining pattern of p63 with calponin

P63	Calponin			Chi sq	p	kappa	p
	Weak staining	Strong Positive	Total				
Weak staining	6	35	41	30.76	0.0001	-0. 597	0.0001
Intense staining	17	2	19				
Total	23	37	60				

The staining pattern of p63 and calponin showed negative correlation with a negative kappa value with a significant “p” value of 0.0001.

COLOR PLATES

1. Gross photograph of adenocarcinoma of stomach

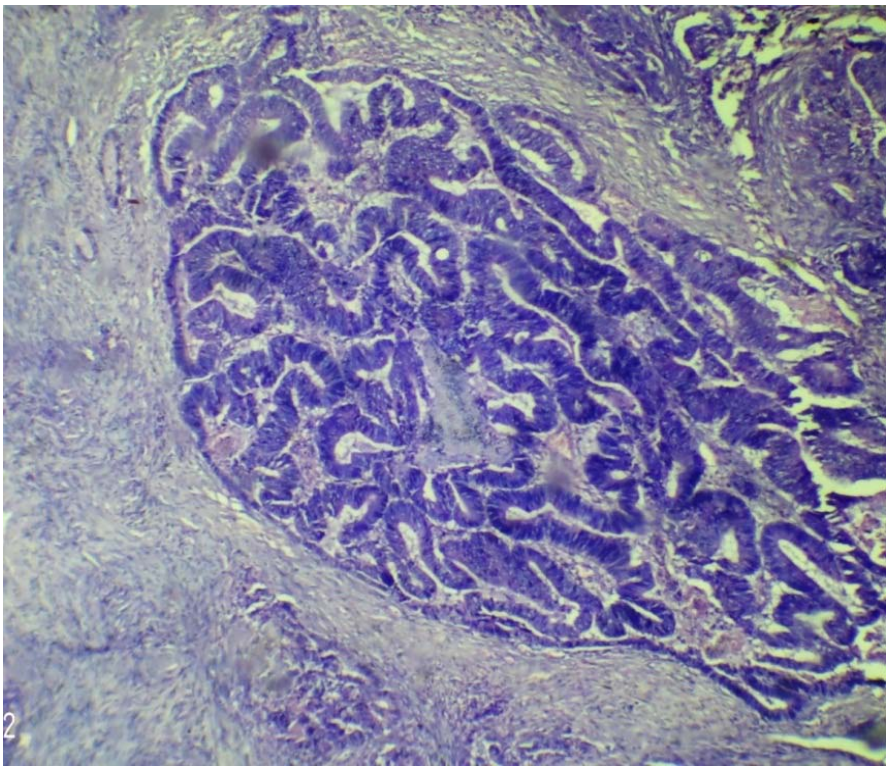
2. Gross photograph of infiltrating adenocarcinoma of stomach

3.Gross photograph of Ileocaecal growth in Ileocolectomy specimen

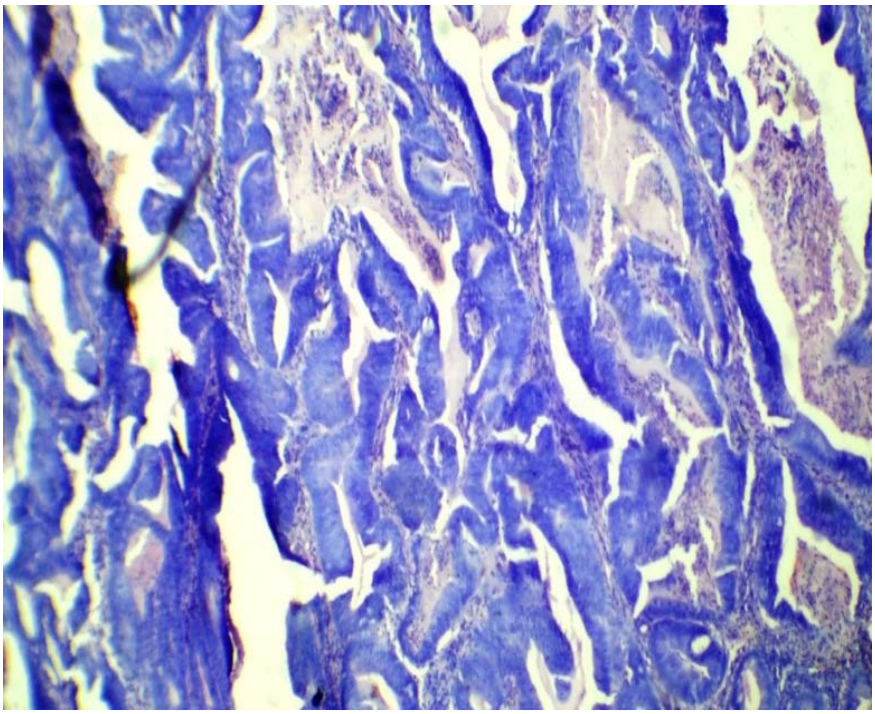
4. Gross photograph of adenocarcinoma of rectum

H& E images

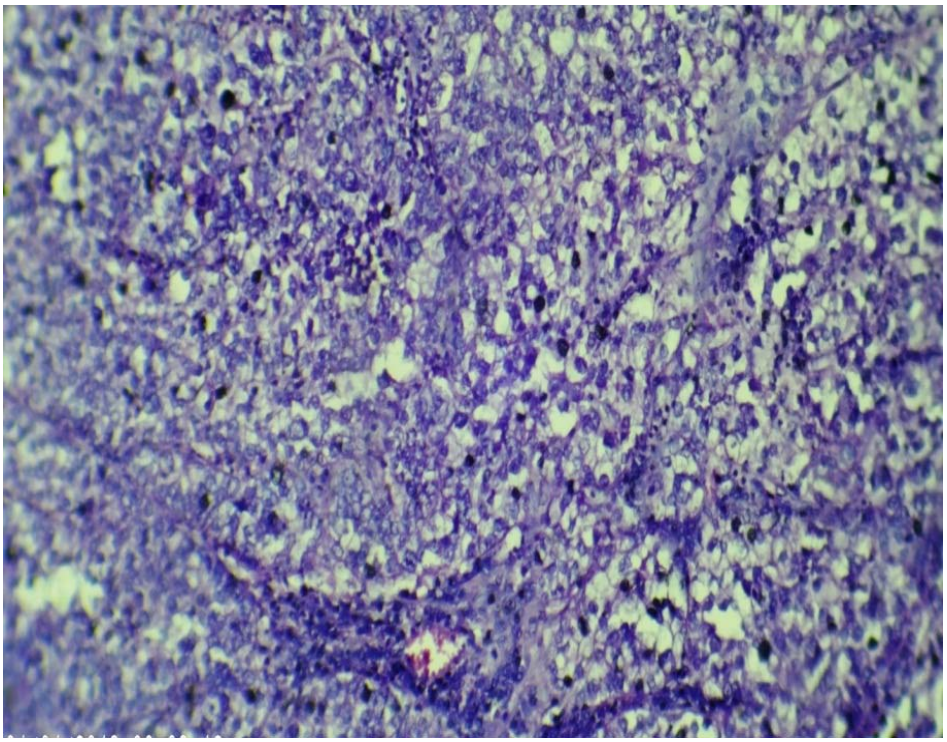
5. Well differentiated adenocarcinoma of stomach (10x)



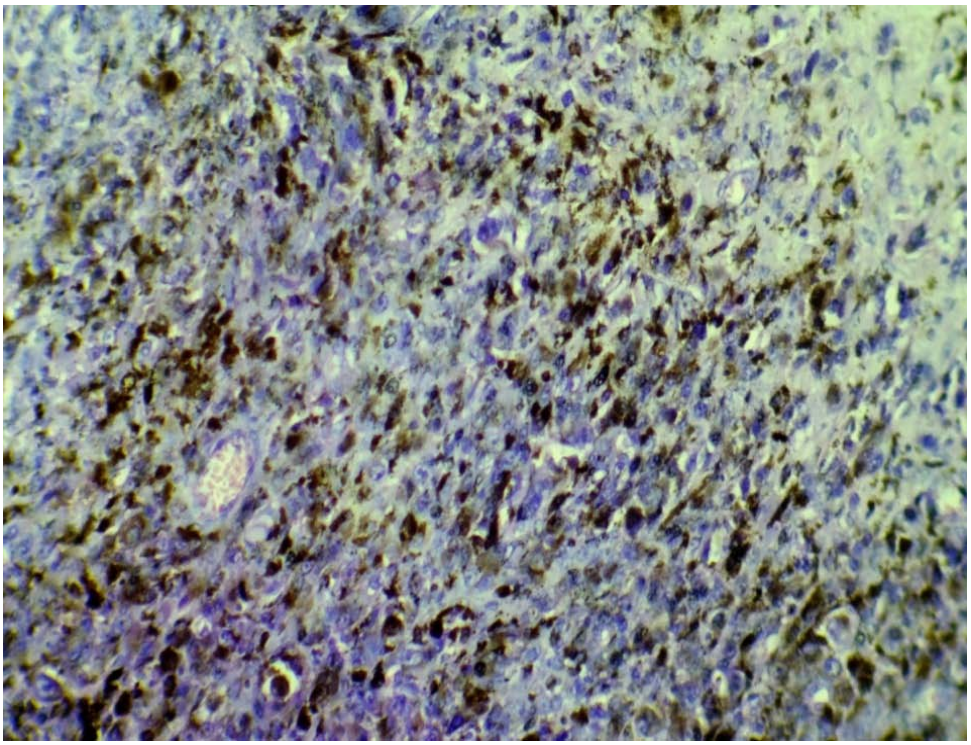
6. Mucinous adenocarcinoma of colon (10x)



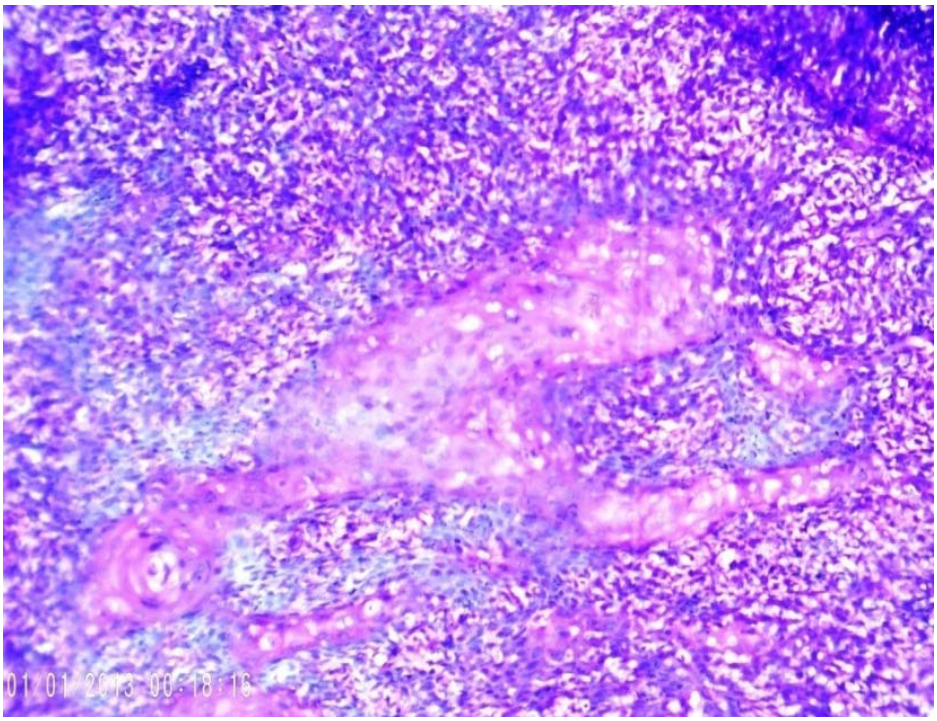
7. Poorly differentiated adenocarcinoma of stomach(10x)



8. Malignant melanoma of anorectum (10x)



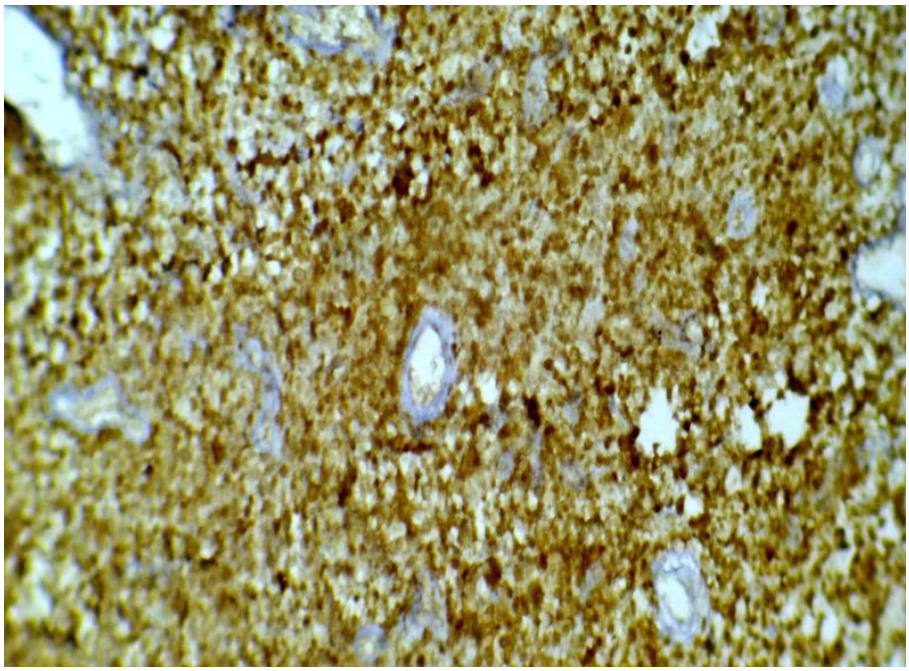
9. Infiltrating squamous cell carcinoma of esophagus (10x)



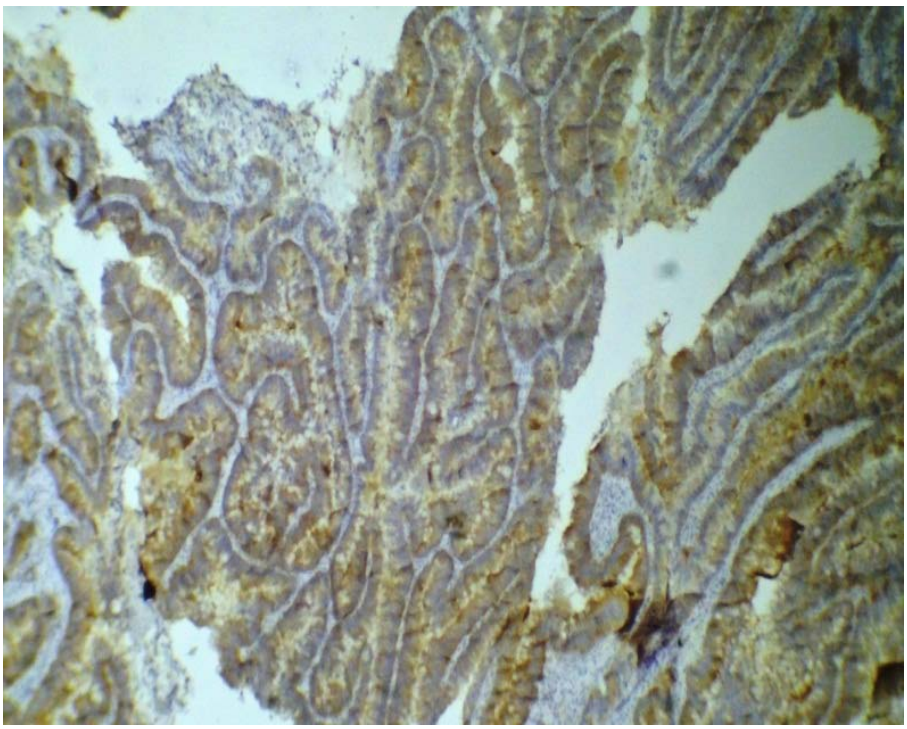
IMMUNOHISTOCHEMISTRY IMAGES

P63

10. Intense staining of P63 in poorly differentiated gastric carcinoma

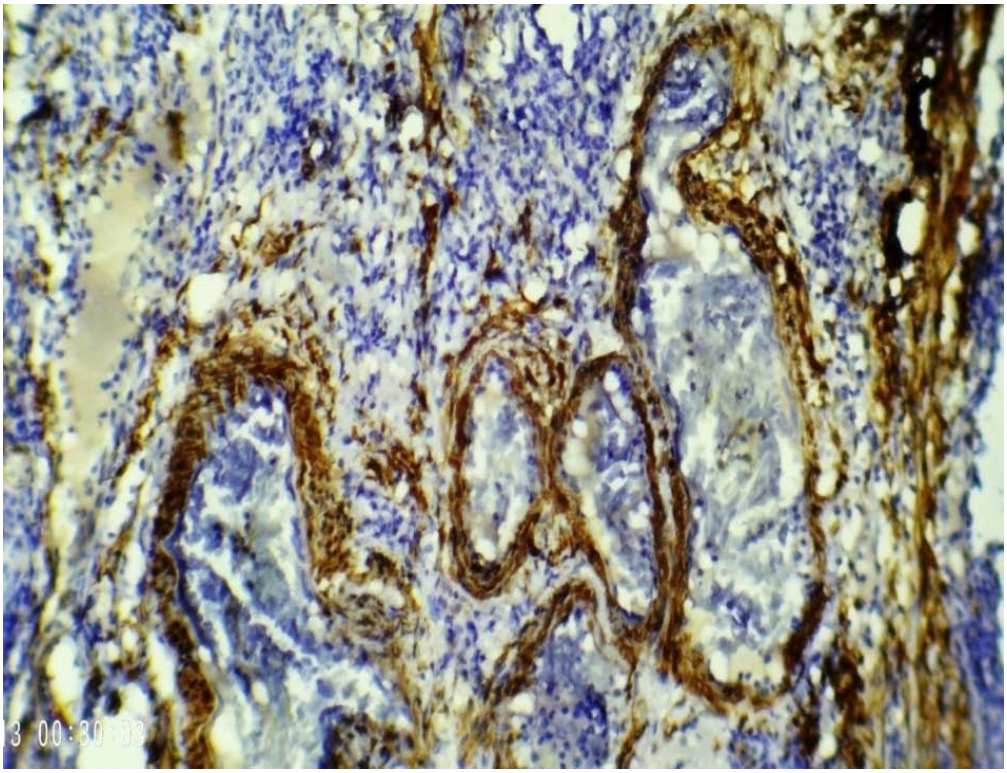


11. Weak staining of P63 in well differentiated adenocarcinoma of stomach



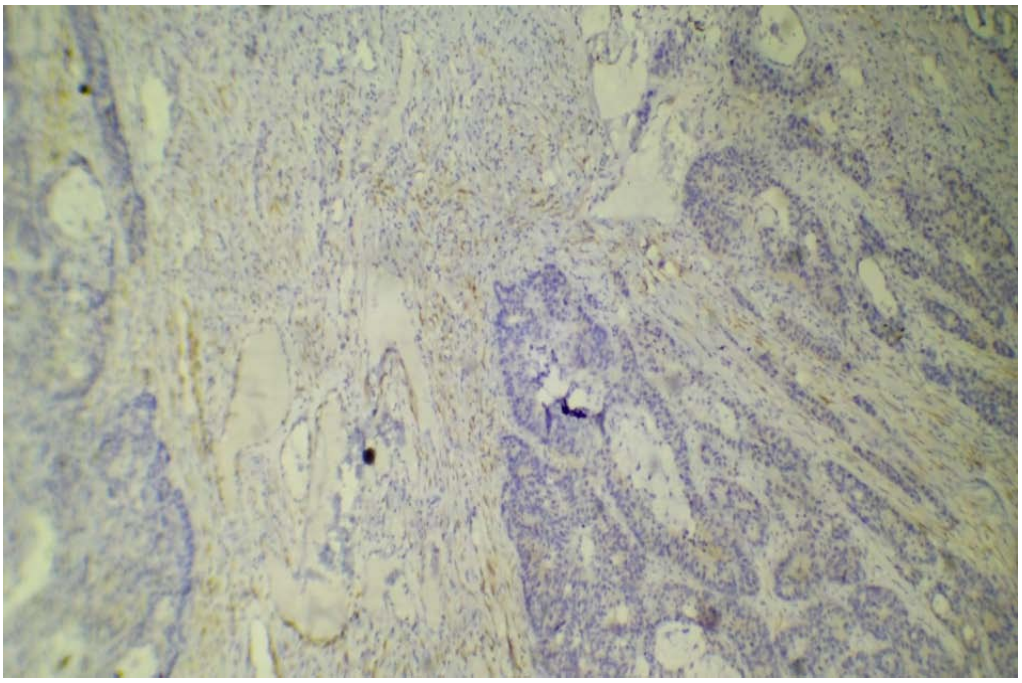
CALPONIN STAINING

12. Intense staining of calponin around tumor blood vessels in well differentiated adenocarcinoma of colon(40x)



13. Weak staining of calponin in poorly

differentiated adenocarcinoma of colon (10x)



DISCUSSION

Gastrointestinal malignancies are increasing in incidence due to various risk factors and life style modifications in our country. Gastrointestinal malignancies are seen in wide range of age group but more common in the age group of 50-60 years of age. In our study, most common age group was found to be in 6^{th} to seventh decade of age group constituting 26 cases (35% of total). In a study conducted by Chhand Das et al(87), they found that most common age group was 50 – 60 years for gastric malignancies and 60 – 70 years for large intestinal carcinomas. In another study conducted by Biren J. Parikh et al (88), they found that peak incidence of gastrointestinal malignancies was around sixth decade. similar results was observed by Leena devi et al and Assem O et al. but the studies conducted by Prabakar et al showed peak incidence was around 5^{th} decade.in a study conducted by Kontham Praveen et al(89), the most common age group was found to be around sixth to seventh decade for all gastrointestinal tract malignancies.

Table 14: Comparison of age group with other studies

Study	Most common age group
Our study	6^{th} to 7^{th} decade
Chhand Das et al,	6^{th} to 7^{th} decade
Biren J. Parikh et al,	6^{th} decade
Prabakar et al	5^{th} decade
Kontham Praveen et al	6^{th} to seventh decade

In general, the incidence of gastrointestinal carcinomas found to be more in males compared to females, the ratio being 2.5 to 3: 1.In our study, male to female ratio was found to be around 2.33:1compared with study conducted by Chhand Das et al, they found the male to female ratio was around 2.46:1, which is similar with our study. In another study conducted by Biren J. Parikh et al, it was found to be around 2.03:1 which is less compared to our study. Male preponderance was also seen in other studies conducted by Prabakar et al, Mohammad et al and Shahid jamal et al. In a study by Kontham Praveen et al, male to female ratio was found to be around, 1.04 :1.This clearly indicates that incidence of gastrointestinal tract malignancies was found to be more in males compared to females. It may be due to the fact that males are exposed to more risk factors compared to females.

Table 15: comparison of Sex ratio with other studies

study	Male : female
Present study	2.33:1
Chhand Das et al	2.46:1
Biren J. Parikh et al	2.03:1
Kontham Praveen et al	1.04 :1

In our study, most common site involved is stomach constituting 22 cases (36.7% of the total cases). In a study conducted by Chhand Das et al, similarly gastric lesions found out to be the most common lesions, constituting appromately 50% of total cases and least common site was esophagus constituting 2.6% of total cases. Least common site in our study was found to be ileum(one case) and esophagus(two cases) constituting 5% of total cases. In our study, rectum is the second most common site (16 cases) and colon is the third most common site (9 cases). If we take large intestinal carcinomas as a whole, combining colon and rectal carcinomas as single entity as colorectal carcinomas, then it will become the most common site constituting 25 cases (41.7% of total cases). In an another study by Biren J. Parikh et al, the commonest site was found to be colorectal carcinomas (38.4%), similarly in the studies conducted by shahid Jamal et al and Thomas et al was found to be 45.23% and 79.67% respectively. In a study by by Kontham Praveen et al, they have taken 108 gastrointestinal tract biopsies, the most common lesion in their study was large intestinal lesions, constituting 37.22% of the total cases.

Table 16: Comparision of most common site of involvement with other studies

Study	Most common site
Present study	Gastric carcinomas (36.7%)
Chhand Das et al,	Gastric carcinomas (50%)
Biren J. Parikh et al	Colorectal carcinomas (38.4%)
Shahid Jamal et al	Colorectal carcinomas (45.23%)
Kontham Praveen et al,	Colorectal carcinomas (37.22%)

In general, adenocarcinomas are most common histopathological entity in gastrointestinal tract malignancies ranging from 70 to 90% of the total gastrointestinal tract malignancies.

In our study, the most common histopathological diagnosis was found to be adenocarcinoma constituting 53 cases (88.4% of the total cases) with one case of intestinal type of adenocarcinoma and 5 cases of mucinous adenocarcinomas. The least common histopathological diagnosis was found to be adenosquamous carcinoma and malignant melanoma, each constituting 2cases (3.3% of the total cases). The study conducted by Chhand Das et al, the most common histopathological diagnosis was found to be adenocarcinomas constituting 91.67% which is similar with our study. In their study they had included lymphomas and they found that non hodgkins lymphoma is the second most common histopathologial diagnosis followed by GIST. In an another study conducted by Biren J. Parikh et al, they found that the most

common histopathological type was adenocarcinomas constituting 45.68%. followed by squamous cell carcinoma constituting around 32.10%.

Table 17 :Comparison of histopathological type with other studies

Study	Most common histopathological type
Present study	Adenocarcinoma (88.4%)
Chhand Das et al	Adenocarcinoma (91.67%)
Biren J. Parikh et al	Adenocarcinoma (45.68%).

In general, the most common grade in gastrointestinal malignancies was found to be well differentiated. In our study the most common histopathological grade was moderately differentiated carcinomas, constituting 22 cases (36% of the total).In a study conducted by kontham Praveen et al, well differentiated carcinomas constitute the most common grade constituting around 57.14% of the total cases.

In our study, we had evaluated the role and prognostic significance of p63 and calponin among those sixty cases. We have described the staining pattern of P63 as weak and intense staining depending upon the nuclear positivity of tumor cells and we compared the staining pattern with lymph node status available among those sixty cases.

In our study, P63 staining pattern was correlated with histopathological grading among those sixty cases. Out of these sixty cases, 17 cases of poorly differentiated carcinomas showed intense staining pattern for P63 and 20 cases

of well differentiated carcinomas show weak staining of P63 and revealed a statistically significant “P” value of 0.001. These results are compared with study by Y.Song et al, it also showed significant “P”value of 0.017.

Among the sixty cases, lymph node positivity was found in 17 cases, of these 17 cases, 12 cases(< 3 positive lymph nodes) showed weak staining of P63 and remaining 5 cases(> 3 positive lymph nodes) showed intense staining of P63. Lymph node negativity is seen in 9 cases, in these 8 cases showed weak staining of p63 with a “p” value of 0.29 in this study. This clearly shows that P63 staining pattern is directly related to the lymph node status. in lymph node negative patients, p 63 staining is weak and intense staining is found in poorly differentiated carcinomas. In a study conducted by Y.Song et al(90) on 101 gastric cancer patients. They found out that, there is significant correlation exists between p 63 staining pattern and lymph node status similar to our study with a “P” value of 0.003. Cases with <3 nodes positive showed weak positivity and cases with >3 nodes positive showed strong positivity in their study.

These findings suggest that expression of immunohistochemical marker P63 correlated well with the lymph node status and grade of the gastrointeastinal carcinomas.

Similarly, the staining pattern of calponin was also studied in our study, we found that out of the total sixty cases, intense calponin staining was found in 37 cases (61.7%) and weak staining was found in 23 cases (38.3%).

When compared with the histopathological grading the “p” value is found to be significant with value of 0.01. When compared with the lymph node status, the expression of calponin showed intense staining (strongly positive) in all 9 lymph node status negative patients with a significant “P” value of 0.03. Poorly differentiated carcinomas showed weak staining with calponin. This clearly shows that the expression of calponin is inversely related to the lymph node status and grade of the tumor. In a study conducted by Y. Yanagisawa et al(91), they found the expression of calponin and VEGFR (vascular endothelial growth factor receptor) in tumor blood vessels of 56 colon cancer patients who underwent colectomy. They found that expression of calponin and VEGFR was inversely related to tumor angiogenesis, invasion and metastases. The downregulation of calponin is correlated well with the upregulation of VEGFR in human colon cancer blood vessels. They calculated the CNPV ratio (calponin positive vessel ratio),, the CNPV is more for well differentiated carcinomas and less for poorly differentiated carcinomas. Also CNPV ratio is more for cases with lymph node negative status and less for patients with lymph node matastases with a significant “P” value of <0.05.

From our study it is clearly shown that the expression of immunohistochemical markers P63 and Calponin are inversely related. The intense staining of P63 is correlated well with that of weak staining of Calponin in poorly differentiated gastrointestinal carcinomas with a significant “P” value

when compared, both P63 and Calponin with lymph node status and grade of the tumors.

Our study also signifies that, mutation of tumor suppressor gene P63 leads to tumor progression and reduction of calponin around tumor blood vessels and formation of immature blood vessels leading to pericyte leakage and results in tumor angiogenesis, tumor invasion and metastases.

SUMMARY

In our study we took 60 cases of gastrointestinal tract malignancies. out of these 60 cases 33 cases were biopsies(endoscopic biopsies from esophagus, stomach, colon, rectum and anus) from various anatomical sites of carcinomas of the gastrointestinal tract and 27 cases were gastrointestinal tract malignancy specimens (gastrectomy specimens including partial, distal,subtotal and total gastrectomies, colectomy specimens including hemicolectomy, extended hemicolectomy and Abdomino perineal resesction(APR)specimens) were included in our study. Our study period was from June 2014 to June 2017. In our study we excluded accessory organs of gastrointestinal tract, liver and pancreas and also appendix as no case of malignancy of appendix was reported during our study period. In this study only gastrointestinal tract carcinomas was studied and we had excluded other malignancies of gastrointestinal tract like lymphomas, sarcomas and Gastrointestinal tract tromal tumors (GIST).

Of these sixty cases, 52 cases were from our department and remaining 8 cases from our collaborating institution, Govt., Arignar Anna Memorial Cancer Hospital and Research Institute(GAAMCH&RI). All eight cases were specimens received from our collaborating institution after getting proper permission from the Director of the Institution, GAAMC&RI and from our institutional ethical committee.

In our study we found out the significance of age distribution, site distribution, gender distribution, lymph node status and expression of

immunohistochemical markers p63 and Calponin in our sixty cases and compared with other studies done on gastrointestinal tract malignancies. We found the following inferences from our study

The most common age group affected by gastrointestinal tract carcinomas in our study belongs to sixth to seventh decade, constituting 35% of the total cases and the least common age group was < 30 years of age constituting only 2 cases (only 3.3% of the total cases). Males were more affected than females in our study with sex ratio of 2.3:1.

The most common anatomical site of gastrointestinal tract involved in our study was gastric carcinomas constituting 36.7% followed by rectal malignancies constituting 16 % and least common site is ileum and esophagus, together constituting only 5% of the total cases studied. If we take colorectal carcinomas as single entity then it becomes the most commom site constituting 41.7% of the cases.

In our study, the most common histopathological diagnosis among those sixty cases was adenocarcinoma constituting 88% of the total cases. the least common was malignant melanoma constituting 3.3 %. In our study, the most common grade among those sixty cases was found to be moderately differentiated constituting 22 cases (36.7% of total cases). The lymph node status was available for 26 cases in our study,of these 17 cases showed lymph node metastases and 9 cases showed no evidence of lymph node metastases. In this study, the lymph node status correlates well with the histopathological

grade of the tumor. Most of the poorly differentiated tumors showed lymph node metastases.

Immuno histochemical expression of P63 among these 60 cases showed intense staining pattern in 19 cases (31.7%) and weak staining pattern on 41 cases (68.3%).

Out of 9 cases of lymph node negative cases, 8 cases showed weak staining of P63. among 17 lymph node positive cases, 12 cases showed weak staining and 5 cases showed intense staining of P63.

The expression of P 63, when correlated with histopathological grade was found to be very significant, with a significant “P” value of 0.001.Most of the well differentiated tumors showed weak staining of P63.

Similarly, the expression of Calponin was strongly positive in 37 cases (61.7%) and weakly positive in 23 cases (38.3%). The expression of Calponin, when correlated with lymph node status was stastistically significant with a “p” value of 0.03.

The expression of Calponin is correlated with histopathological grading of those 60 cases and it was found to be statistically significant with a “P” value of 0.01. The immunohistochemcial expression of Calponin was found to be inversely related to the lymph node status and histopathological grading of the gastrointestinal tract malignancies studied.

In the present study, the expression of p63 is inversely related to calponin expression in gastrointestinal tract carcinomas.

CONCLUSION

In our study we analysed sixty cases of gastrointestinal tract malignancies and found the expression of immuno histochemical markers p63 and calponin in those sixty cases. Increased expression of p63 is directly related to the lymph node metastases and the grade of the tumors. while calponin expression is inversely related to the grade and lymph node status of the cases studied. This study clearly showed that higher expression of calponin inhibits tumor metastases, tumor angiogenesis and tumor invasion, especially in patients with colorectal carcinomas. So targeted therapy with calponin will be prognostically beneficial to the patients with gastrointestinal tract carcinomas. Similarly p63 targeted therapy will be beneficial and improves the survival rate of the patients with gastrointestinal tract malignancies, especially in poorly differentiated esophageal carcinomas, gastric carcinomas and colorectal carcinomas.

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ANNEXURE - I

PROFORMA

Date:

1. Name : OP/IP No :

2. Age :

3. Sex : Male Female

4. History :

Histopathological grading

Histopathological categorization, pathological staging & grading of Gastro-intestinal carcinomas using Hematoxylin & Eosin stained sections.

Esophageal carcinoma – Grade 1 – 3.

Gastric carcinoma – Grade 1- 3.

Colorectal carcinoma – Grade 1 – 3.

Anal carcinoma – Grade 1 – 3.

Nodal status.

Depth of invasion.

Expression of p63 and calponin in immunohistochemical stained sections

p63 Expression:

POSITVE - Intense Staining – (>50% of tumor cells positive) Poor prognosis.

- Weak staining – (<5% of tumor cells positive) Better prognosis.

NEGATIVE

Calponin Expression

POSITIVE - staining of stromal tumor blood vessels

-Intense staining – Better prognosis.

- Weak staining – Poor prgnosis.

NEGATIVE

ANNEXURE 2

MASTER CHART KEY

1. Sex:

Male – 1

Female -2.

2. Histopathological diagnosis:

Adenocarcinoma -1

Adenosquamous carcinoma- 2

Squamous cell carcinoma – 3

Mucinous carcinoma – 4

Malignant melanoma – 5

Intestinal type carcinoma – 6

3. Histopathological grading of tumors:

Well differentiated tumors – 1

Moderately differentiated tumors – 2

Poorly differentiated tumors – 3.

4 Immunohistochemical markers staining pattern :

- P63 stianing pattern-

Weak staining – 1

Intense staining – 2

- Calponin staining pattern-

Weak staining – 1

Strong positive staining – 2

5. Lymph node status :

Positive – 1

Negative – 2

Not known – 3

6. Specimen type:

Biopsy – 1

Colectomy -2

Abdominoperenial reection – 3

Partial gastrectomy – 4

Distal gastrectomy – 5

Subtotal gastrectomy - 6

Total gastrectomy – 7.

ANNEXURE 3

GLOSSARY

AIN	:	Anal intraepithelial neoplasisa
ATZ	:	Anal transformation zone
CEA	:	Carcino embryonic antigen
CN	:	Calponin
DAB	:	Di amino benzidine
EBV	:	Ebstein barr virus.
EMR	:	Endoscopic mucosal resection
FAP	:	Familial adenomatoses polyposis
GIT	:	Gastrointestinal tract
GERD	:	Gastroesophgeal reflux disease.
GALT	:	Gut associated lymphoid tissue
H&E	:	Haematoxylin & Eosin
HNPCC	:	Hereditary non polyposis colon cancer
HDI	:	Human Development Index
HPV	:	Human papilloma virus.

ICC	:	Interstitial cells of cajal.
IHC	:	Immunohistochemistry
PBS	:	Phosphate buffer solution
PTD	:	Pericolonic tumor deposits.
SCJ	:	Squamocolumnar junction.
TBS	:	Tris buffer solution
WHO	:	World Health Organisation

Master Chart

Sl. No	Path no	Age	sex M/F	Site	clinical diagnosis	Histopathological diagnosis	HPE grade	P 63	Calponin	specimen type	lymph node status
1	1664/15	48	2	Anorectum	anorectal growth	3	2	1	2	1	3
2	36/16	55	1	Anus	anal growth	1	2	1	2	1	3
3	1880/16	58	1	Anus	anal growth	3	2	1	1	1	3
4	406/15	55	1	Anus	growth anal canal	4	2	1	1	1	3
5	1375/15	70	1	Anus	Anal carcinoma	1	1	1	2	1	3
6	548/15	52	1	Anus	growth anal canal	5	3	1	1	1	3
7	141 /15	47	1	Anus	Anal polyp	2	2	1	2	1	3
8	133/15	65	2	Anus	anal canal growth	3	2	1	2	1	3
9	1145/15	60	1	Colon	carcinoma asc.colon	1	1	1	2	2	1
10	962/17	57	1	Colon	carcinoma colon	1	2	1	2	2	2
11	432/17	42	2	Colon - descending colon	growth descending colon	1	2	2	2	2	2
12	498/17	75	2	Colon - splenic flexure	carcinoma splenic flexur	1	1	1	2	2	2
13	877/17	54	1	Colon transverse	growth transverse colon	1	1	1	2	1	3
14	210/17	40	1	Colon transverse	mass transverse colon	1	2	1	2	2	2
15	290/17	60	1	Esophagus	carcinoma esophaus	2	3	2	1	1	3
16	1409/15	42	1	Ileum	carcinoma intestine	1	2	1	2	1	3
17	233/17	20	1	Ileum	intestinal obstruction	1	1	1	2	1	3
18	944/17	55	1	Rectosigmoid region	rectosigmoid growth	5	3	1	1	3	1
19	1019/17	57	1	Rectosigmoid region	rectosigmoid growth	4	1	1	2	2	2
20	631/k/15	70	1	Rectosigmoid region	growth rectosigmoid	1	1	1	2	3	1
21	579/k/16	60	1	Rectosigmoid region	carcinoma rectosigmoid	1	1	1	2	3	1
22	632/k/16	42	2	Rectosigmoid region	growth rectosigmoid	1	1	1	2	2	1
23	1016/17	70	1	Rectum	rectal growth	1	1	1	2	3	2
24	781/15	54	1	Rectum	carcinoma rectum	4	2	1	2	3	1
25	46/16	36	1	Rectum	carcinoma rectum	1	2	1	1	3	3
26	1842/15	36	1	Rectum	carcinoma rectum	1	2	1	2	1	3
27	1866/16	70	1	Rectum	growth rectum	1	1	1	2	1	3
28	771/17	55	1	Rectum	rectal growth	5	3	1	1	1	3
29	626/16	50	1	Rectum	rectal growth	1	2	2	2	1	3
30	45/16	60	2	Rectum	rectal growth	1	2	1	2	1	3
31	67/16	80	1	Rectum	carcinoma rectum	1	2	1	2	1	3
32	901/k/15	60	1	Rectum	carcinoma rectum	1	1	1	2	2	1
33	118/17	68	1	Rectosigmoid	rectosigmoid growth	1	1	1	1	2	1
34	1687/16	65	2	Sigmoid colon	growth sigmoid colon	1	1	1	2	1	3
35	1685/16	68	1	Sigmoid colon	growth sigmoid colon	1	2	1	1	2	1
36	399/17	34	1	Sigmoid colon	growth sigmoid colon	1	1	1	2	2	2
37	410/17	60	1	Stomach - body & antrum	growth body of stomach	1	3	2	1	1	3
38	1776/15	65	2	Stomach- body &antrum	carcinoma stomach	1	3	2	1	7	1
39	734/15	65	1	Stomach antrum	GOO	4	2	1	2	4	1
40	1006/17	70	1	Stomach antrum & pyloru	GOO	1	3	2	1	1	3
41	408/17	55	1	Stomach antrum & pyloru	antral growth	1	1	1	2	1	3
42	994/17	67	2	Stomach antrum & pyloru	antral growth - growth	1	3	2	1	1	3
43	263/k/15	30	2	Stomach antrum & pyloru	carcinoma stomach	1	3	2	1	5	1

Sl. No	Path no	Age	sex M/F	Site	clinical diagnosis	Histopathological diagnosis	HPE grade	P 63	Calponin	specimen type	lymph node status
44	96/16	81	1	Stomach antrum & pyloru	carcinoma stomach	1	3	2	1	4	1
45	422/17	68	1	Stomach antrum & pyloru	pyloric antrum growth	1	1	1	2	6	2
46	709/k/15	57	2	Stomach antrum & pyloru	antral growth	1	1	1	2	6	1
47	1888/15	70	2	Stomach body	growth body of growth	1	3	2	1	1	3
48	1378/15	65	1	Stomach body	GOO	1	3	2	1	1	3
49	1073/15	76	1	Stomach antrum & pyloru	pyloric growth	1	3	2	1	1	3
50	1755/15	67	2	Stomach antrum & pyloru	carcinoma stomach	1	3	2	1	6	1
51	149/16	70	1	Stomach antrum & pyloru	antropyloric growth	1	3	2	1	1	3
52	355/16	45	1	Stomach antrum & pyloru	pyloric antral ulcer	1	2	1	2	1	3
53	255/15	45	1	Stomach pylorus	carcinoma stomach	6	2	1	2	1	3
54	954/17	70	1	Stomach pylorus	pyloric growth	1	3	2	1	1	3
55	294/16	77	1	Stomach pylorus	GOO	1	2	1	2	1	3
56	36/17	70	1	Stomach pylorus	pyloric growth	1	1	1	2	1	3
57	764/16	53	2	Stomach pylorus	carcinoma stomach	1	3	2	1	1	3
58	1121/k/16	62	2	Stomach pylorus	pyloric growth	1	2	1	2	5	1
59	236/k/15	55	2	Stomach pylorus	carcinoma stomach	1	2	1	2	4	2
60	843/16	65	2	Stomach pylorus	carcinoma stomach	1	3	2	1	6	1